

Texas Species Research IAC Final Report

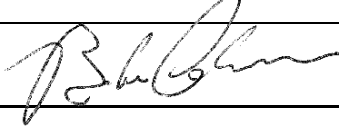
Interagency cooperation contract number (IAC number) 16-5979	University name Texas A&M University
Species name Danaus plexippus	

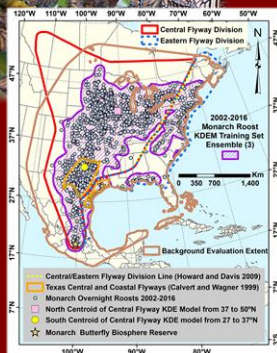
Please attach to this cover sheet to the final research report (narrative format) for this research project. The final report must include but is not limited to the following:

- Introduction;
- IAC Final Report Deliverables;
- Materials, Methods and Quality Control Measures Used;
- Technical Advisory Panel (TAP) Reviews;
- Results/Findings;
- Discussion of Results/Findings (*including how these may be utilized by federal agencies in Endangered Species Act listing decisions*);
- Additional Research Needs (*if any*);
- Literature Review;
- Literature Cited; and
- Other Items of interest.

Please note: Prior to close-out of the IAC, the contractor is required to meet with the Economic Growth and Endangered Species Division .

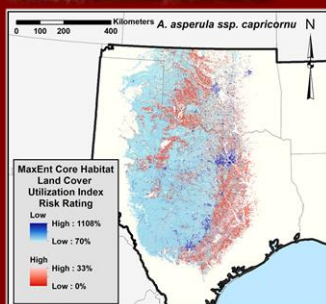
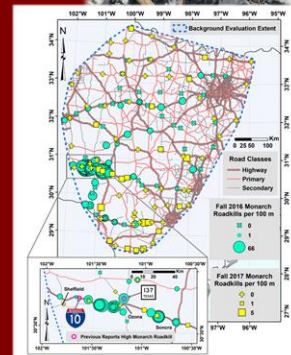
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I certify that the provided information is true and correct to the best of my knowledge based on diligent inquiry.		
Print name Robert N. Coulson	Title Professor, Dept Entomology, Texas A&M Univ	
		Date 31 December, 2018



Investigations on Monarch Butterflies (*Danaus plexippus*) in Texas Using a Spatially Explicit Functional Connectivity Approach

Final Report
IAC #16-5979
Texas Comptroller of Public Accounts
31 December, 2018

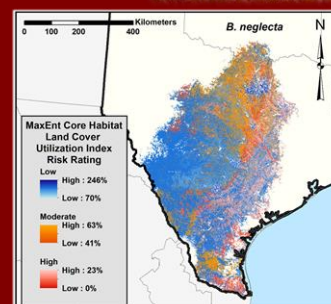
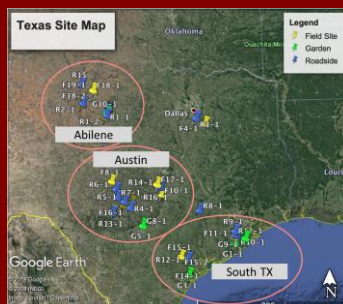


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5th Generation/Winter
Breeding Study Sites



TEXAS A&M
AGRILIFE
RESEARCH

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INTRODUCTION

The overwintering population of eastern migratory monarch butterflies (*Danaus plexippus*) has declined by 82% over the last 23 years. This population trend can be fit to an exponential curve with an annual decline of 7.21% ($y = ae^{bx}$ Fig. 1) ($P = 0.0009$; adjusted $R^2 = 0.486$; ZunZun.com 2018). A concave exponential curve represents the most serious form of species population decline, indicating constant proportional negative pressure on the population (Di Fonzo et al. 2013). Continuance of the 7.21% rate of decline would result in an average of 0.24 ha of overwintering monarchs occurring in 29 years (by 2046-2047), greatly increasing the chance of extirpation of eastern migrating monarchs by an extreme winter storm mortality event as the overwintering population crosses below the 0.25 ha quasi-extinction threshold (Semmens et al. 2016). A total reversal of the 7.21% annual monarch decline, coupled with an annual 7.21% population increase (net change 14.4%), would be needed to restore the size of the overwintering population to current conservation goal of six hectares (Thogmartin et al. 2017) over the next 15 years. A better understanding of hazards and risks for monarchs in the South-Central US are needed to better inform conservation measures in this area that can contribute to a reversal in the long-term 7.2% annual exponential decline in monarch populations.

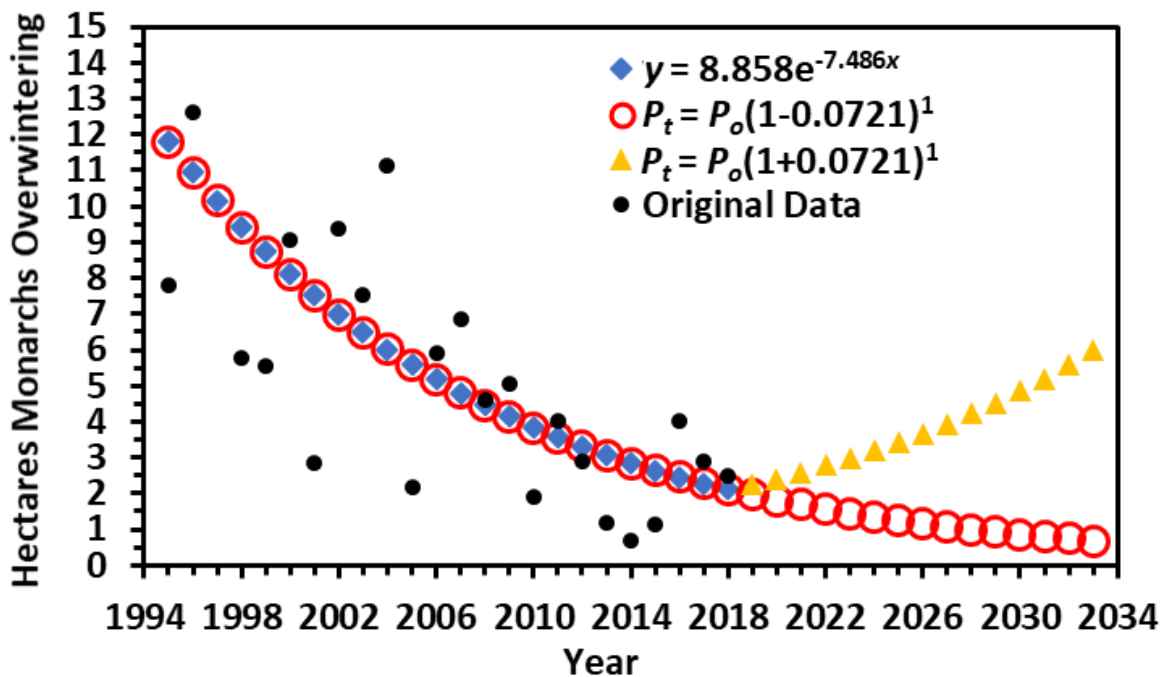


Figure 1. Annual monarch population in hectares in Mexican overwintering sites from 1995 to 2018 (original data, black circles; Vidal and Rendón-Salinas et al. 2014; Monarch Watch 2018) with fitted exponential curve, $y = ae^{bx}$ (adjusted $R^2 = 0.49$; $P = 0.00009$; blue diamonds), and corresponding geometric population growth equation curve, $P_t = P_o(1 + r/n)^{nt}$, where P_t is the final hectares (2.11), P_o is the initial hectares (11.79), t is the number of years (23), n is the number of sub-periods (1), and r is the population growth (or declination) rate (derived population declination of 7.21% per year; open red circles). Fifteen years to restore 6 ha of overwintering monarchs based on totally reversing the current decline to 7.21% growth per year (gold triangles).

Identifying core migratory pathways, breeding habitats, and associated threats is important for developing conservation priorities for declining migratory species (Hardesty-Moore 2018; Runge et al. 2014), including the eastern monarch butterflies. This report develops the first models of the core fall migratory pathways for the eastern monarch and spatially identifies associated threats. Two spatial modeling methodologies are used in and compared defining the fall migration paths, interpolative kernel density estimate models (KDEMs) and MaxEnt ecological niche models. Hazards identified along the core migratory pathways include roadkill, mosquito ultra-low volume (ULV) spraying, and high usage of glyphosate herbicides and neonicotinoid insecticides. A more detailed study is made of roadkill, which is the foremost migratory threat along the monarch Central Flyway in West Texas. Spatial MaxEnt ecological niche models are used in projecting and estimating monarch roadkill across the core migratory pathway from Oklahoma to Texas and northern Mexico. Milkweed and nectar resources in the south-central US (Texas, Oklahoma, Louisiana, and Arkansas) are critical for the spring and fall monarch migrations. We developed ecological niche models for the four major native milkweeds and ten major nectar plants (including fall and spring species) in the South-Central US. These niche models were then used with land cover classifications to develop a land cover risk assessment for each plant resource. The land cover utilization index was developed for this purpose, representing the estimated utilization by a plant species of each land cover type within the modeled core niche of each plant. We also investigated the poorly known general seasonal occurrence of monarch larvae and their use of different milkweed resources across different phenoregions of the South-Central US. Pre-migrant adults arriving from the North in late summer to the South-Central region prior to the main migration lay eggs which give rise to the fall 5th generation monarchs. The 5th generation can significantly contribute to the monarch fall migration, but little is known about its distribution, host plant use, and natural enemy impacts. We developed three-dimensional spatio-temporal KDEMs that distinguish where and when the pre-migrants and migrants occur by the week of year. Field surveys in Texas examined habitat and milkweed use by 5th generation and wintering monarch larvae, as well as the impacts of natural enemies such as the pathogen *Ophryocystis elektroscirrha* (OE) and parasitoid tachinid flies. Progeny from the 5th generation and corresponding field-caught adults were tagged to potentially assess their contribution to the number of overwintering monarchs in Mexico from tagged recoveries there.

Di Fonzo M, Collen B, Mace GM (2013) A new method for identifying rapid decline dynamics in wild vertebrate populations. *Ecology and Evolution* 3:2378-2391.

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IAC FINAL REPORT DELIVERABLES

Each deliverable is in a separate section of the report, with the last being the *Literature Review*. The sections for each of the first nine deliverable are subdivided into the subheadings of *Materials, Results, Discussion, and References*. The results and discussion sections focus on results related to Texas, although some studies had larger geographic scopes.

Section I. Monarch fall migration model and spatial identification of hazards.

Section II. Monarch fall roadkill model and risk assessment.

Section III. Monarch milkweed habitat models and land cover risk assessment.

Section IV. Monarch larval seasonality and host plant use by phenoregions.

Section V. Monarch spring and fall nectar plant habitat models and land cover risk assessment.

Section VI. Monarch weekly pre-migrant/migrant models.

Section VII. Habitat use by pre-migrant monarch butterflies (and their 5th generation offspring).

Section VIII. Impact of *Ophryocystis elektroscirrha* (OE) and tachinid flies.

Section IX. Contribution of 5th generation monarch butterflies to the overwintering population.

Literature review of monarch butterfly population status and trends.

MONARCH BUTTERFLY HABITATS, RISK ASSESSMENTS, AND 5TH GENERATION/WINTER BREEDING POPULATIONS

SECTION I: MONARCH FALL MIGRATION MODEL AND SPATIAL IDENTIFICATION OF HAZARDS

Introduction

Many migratory species of conservation concern are declining worldwide (Hardesty-Moore 2018). Comprehensive conservation strategies for these species must take into account breeding, migratory stopover, and overwintering sites. Annual mortality for migrant species, especially birds, can be highest across migratory routes, particularly in narrower portions of migratory pathways. Spatial models of migratory pathways are an important tool for identifying conservation priorities (Runge et al. 2014). Common approaches for defining migratory pathways include kernel density estimation (KDE) (Rayner et al. 2017; Pierce et al. 2017), and correlative ecological niche models, such as MaxEnt (Huff et al. 2012; Williams et al. 2017). These methods have not been thoroughly compared to evaluate their contributions to migratory pathway analyses.

A variety of potential anthropogenic hazards to adult fall migrating monarchs have been proposed (Agrawal and Inamine 2018; Malcom 2018). We focus on four relatively important potential threats identified in the literature. Two threats have been previously reported as mortality agents for fall adult migrating monarchs, roadkill (McKenna et al. 2001; Tracy 2018; see also Baxter-Gilbert et al. [2015]) and ultra-low volume (ULV) spraying of insecticides (e.g., resmethrin and permethrin) for adult mosquito control (Journey North 2000; Oberhauser et al. 2006, 2009; see also Barger [2012]; Hoang and Rand [2015]). The two additional potential threats include reduced fitness from scarcer nectar resources due to increased glyphosate herbicide use on agricultural lands (Brower et al. 2006), and reduced fitness from exposure to agricultural pesticides, particularly systemic neonicotinoids contaminating flower nectar sources near agriculture (Stenoien et al. 2018). Potential sublethal effects of reduced fitness for any of these threats have not yet been demonstrated (Braak et al. 2018; Stenoien et al. 2018). Spatial identification of potential threats is needed along the fall migratory routes, especially along core pathways approaching the overwintering sites (Brower et al. 2006; Badgett and Davis 2015), and it is prerequisite to further migratory risk assessments (e.g., Liechti et al. 2013).

The goals of this study are to compare KDEM and MaxEnt for defining the core fall migration pathways of the eastern monarch, and to identify four potential migratory hazards along these pathways, including roadkill, mosquito ULV spraying, and heavy use of agricultural pesticides of glyphosate herbicide and neonicotinoid insecticides. The specific objectives are to (1) project and compare the eastern monarch fall migratory pathways with the two approaches of MaxEnt and KDEM; (2) examine annual variability in the migration pathways, delineating core migration routes; and (3) spatially identify potential hazards to the fall migration, analyzing implications for monarch conservation planning and future migratory risk assessment.

Methods

Spatial models of the monarch fall migratory pathways were developed from 10 km² resolution 2002-2015 Journey North citizen science data of monarch overnight roosting during fall migrations (Journey North 2017) (Fig. I.1). MaxEnt ecological niche models utilized 80 environmental variables, including climate and land cover indices. The 80 variables were screened using a random subset feature selection algorithm to produce 12 higher performance niche models with six variables each. The KDEM models were strictly interpolative and were represented by KDE intensity surfaces of the roost data that were normalized from zero to one. Three training set KDEMs each were produced for combined years 2002-2015 and for the individual years 2005-2016. In addition, KDEM consensus models were produced for the three 2002-2015 training set KDEMs and for the 12 annual 2005-2016 KDEMs. General Central and Eastern Flyway division migratory pathways were updated and core migratory pathways were identified from the various consensus models. Hazards were spatially identified across the monarch migratory pathways using various data sources for monarch roadkill hotspots (citizen science data and surveys), US counties with mosquito ULV spraying (screened news and governmental websites), and US counties with upper 75th percentile usage of glyphosate herbicides and neonicotinoid insecticides (USGS 2018) (for additional details, see Appendix A, Tracy et al. 2018).

Results

The KDEM migratory pathways best represented patterns of monarch movement towards overwintering locations through less favorable habitats of northern Mexico. MaxEnt models best identified areas potentially suitable for nectar resources in the Great Plains (Fig. I.2). Migratory routes varied as much as 200 km from east to west in the southern Central Flyway (Fig. I.3). The consensus annual KDEM boundary was used to identified core migratory route through the Central flyway, and the southern core funnel pathways for the Central and Eastern Flyways, the Central Funnel and the Coastal Funnel, respectively (Figs. I.4-7).

Seven reports of monarch October/November roadkill hotspots were identified, four along the Central Funnel in Texas (Journey North 2017, Tracy 2018) and three in Mexico (Correo Real 2015) (Table B.5; Figs. I.4A, I.5). In West Texas, two hotspots had 52 and 66 roadkill monarchs per 100 meter along Interstate Highway 10 west of Sonora and Ozona, respectively (Tracy 2018). In Mexico, two hotspots southwest of Monterrey had 250 and 575 roadkill monarchs per 100 m on Mexico Federal Highway 40D (Correo Real 2015).

Four reports of dozens to hundreds of dead adult fall migrating monarchs attributed to mosquito adulticide ULV truck spraying were found from 1999 to 2011. These were located in Central Park, New York (New York Daily News 1999; malathion), Gaylord, Minnesota (Journey

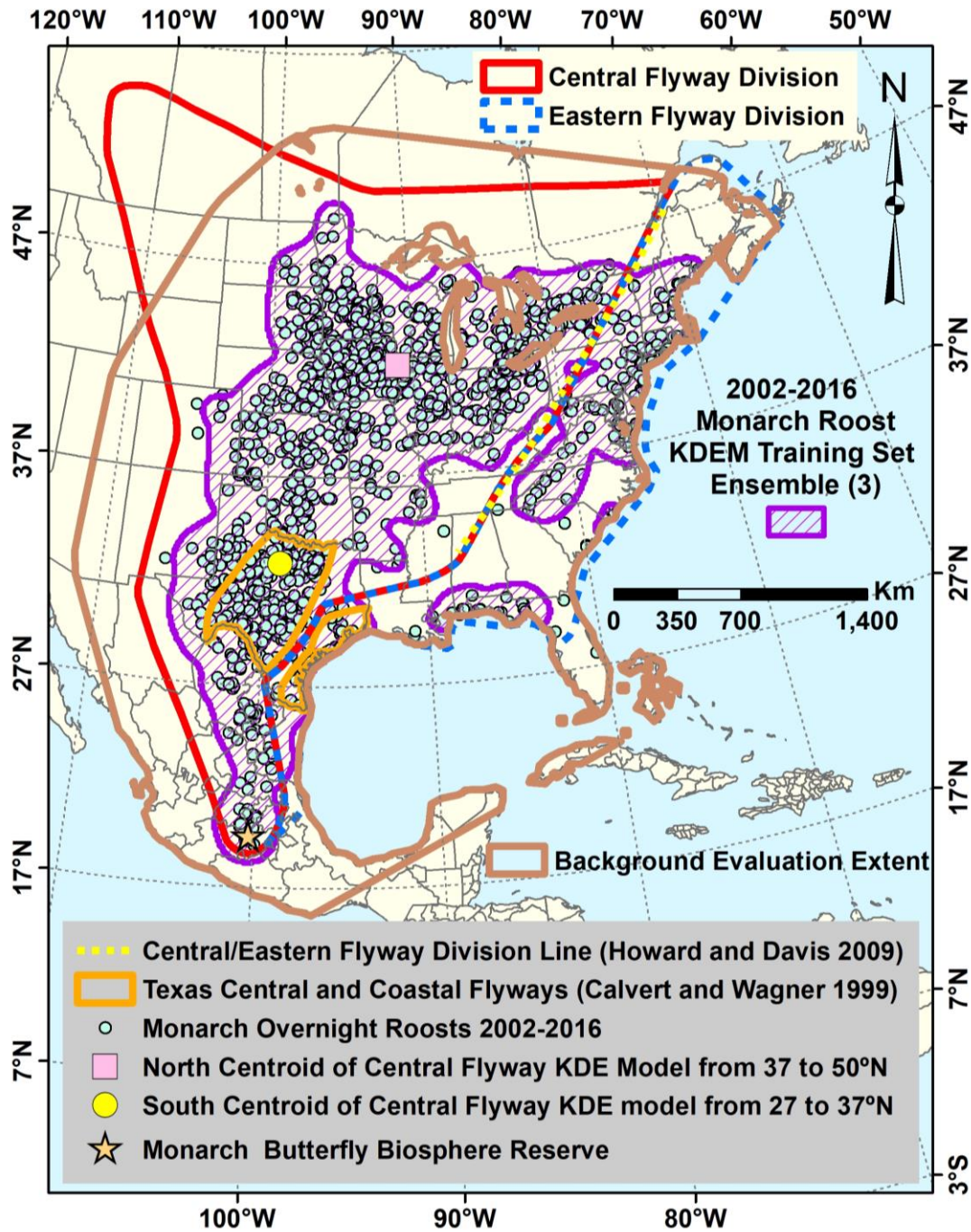


Fig. I.1. Monarch overnight roosts from 2002 to 2016 (Journey North 2017), range of the monarch eastern migratory population, previous and updated monarch flyway divisions, background evaluation extent for model training and testing, migration pathway of minimum consensus kernel density estimation model (KDEM) with north (37-50°N) and south (27-37°N) centroids for the KDEM in the Central Flyway (see Table B.4 for shapefiles).

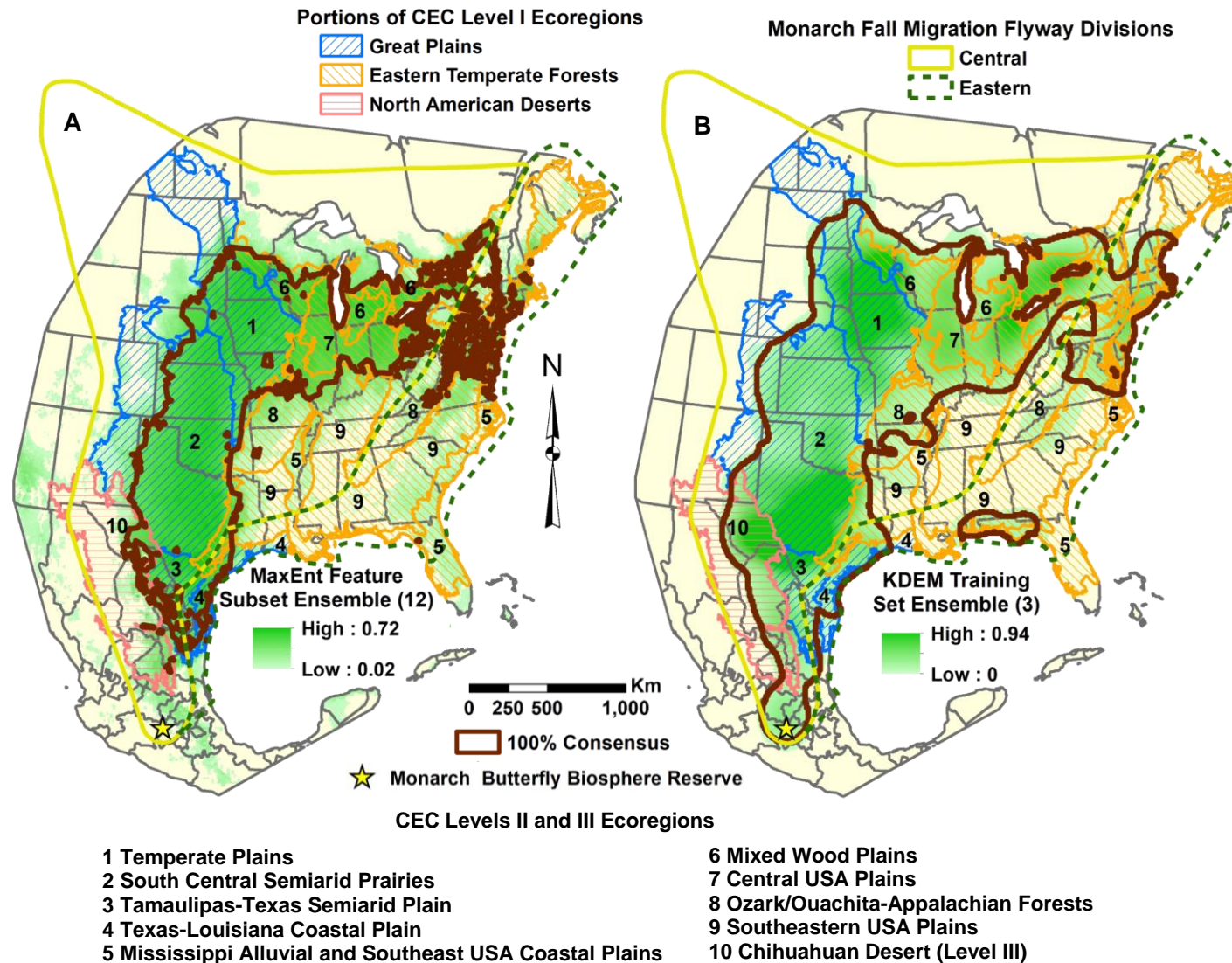


Fig. I.2. Monarch fall migration pathway minimum calibration consensus of average consensus models for 2002–2016, including CEC (2005) ecoregions (including 100% consensus boundaries): (A) MaxEnt feature subset ensemble of 12 models developed from subsets of six of 80 variables by random subset feature selection for high AUC_{psa} ; and (B) kernel density estimation model (KDEM) training set ensemble of three models developed by three-fold training data partition (see Table B.4 for shapefiles; see Figs. I.1 and I.4A for binary minimum consensus KDEM).

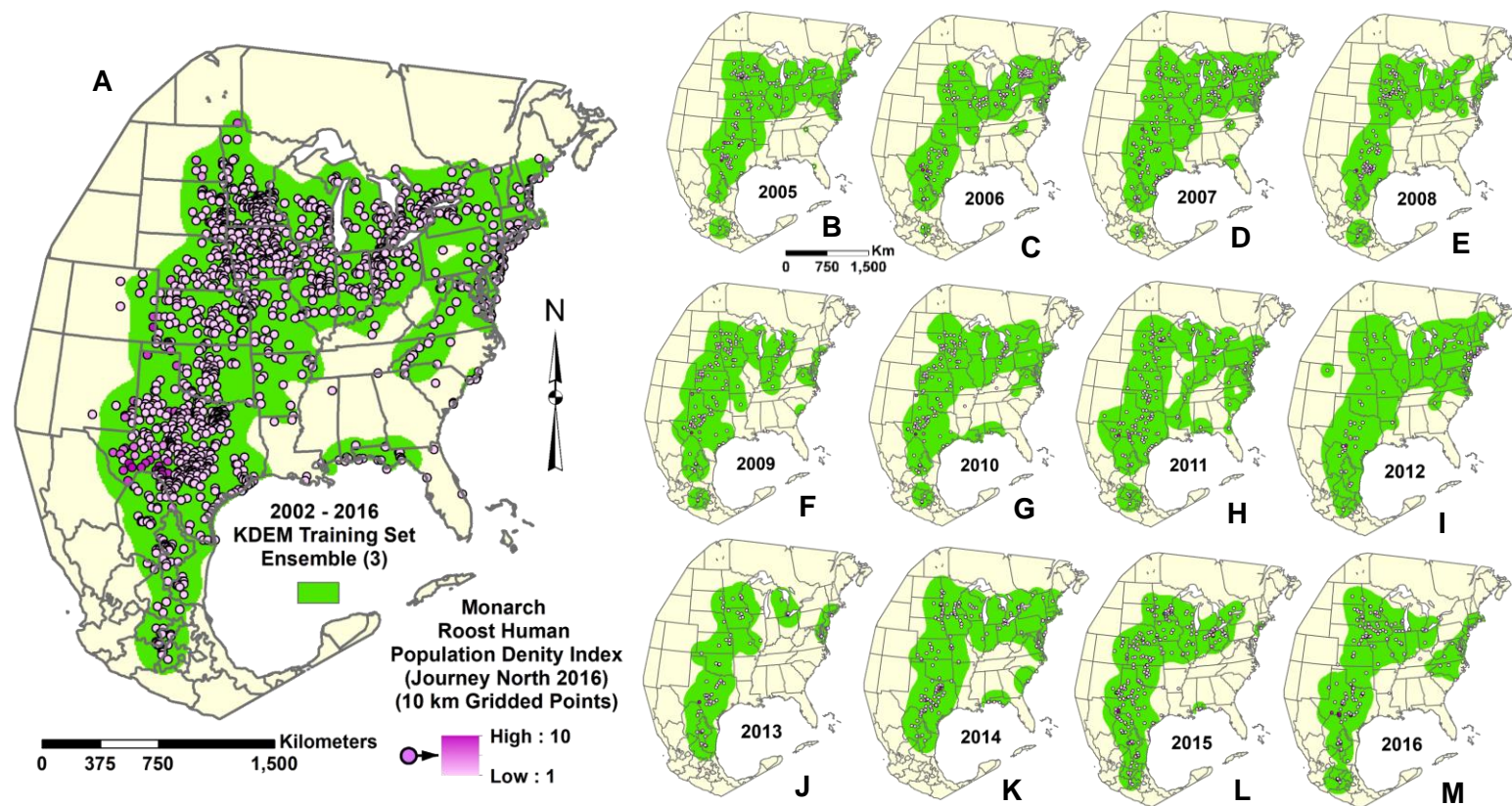


Fig. I.3. Monarch fall migration pathway kernel density estimation models (KDEMs) from (A) 2002–2016 combined data, and (B–M) for each year from 2005 to 2016. Models represent minimum frequency consensus training set ensemble of three binary calibrated models developed from a three-fold training data partition.

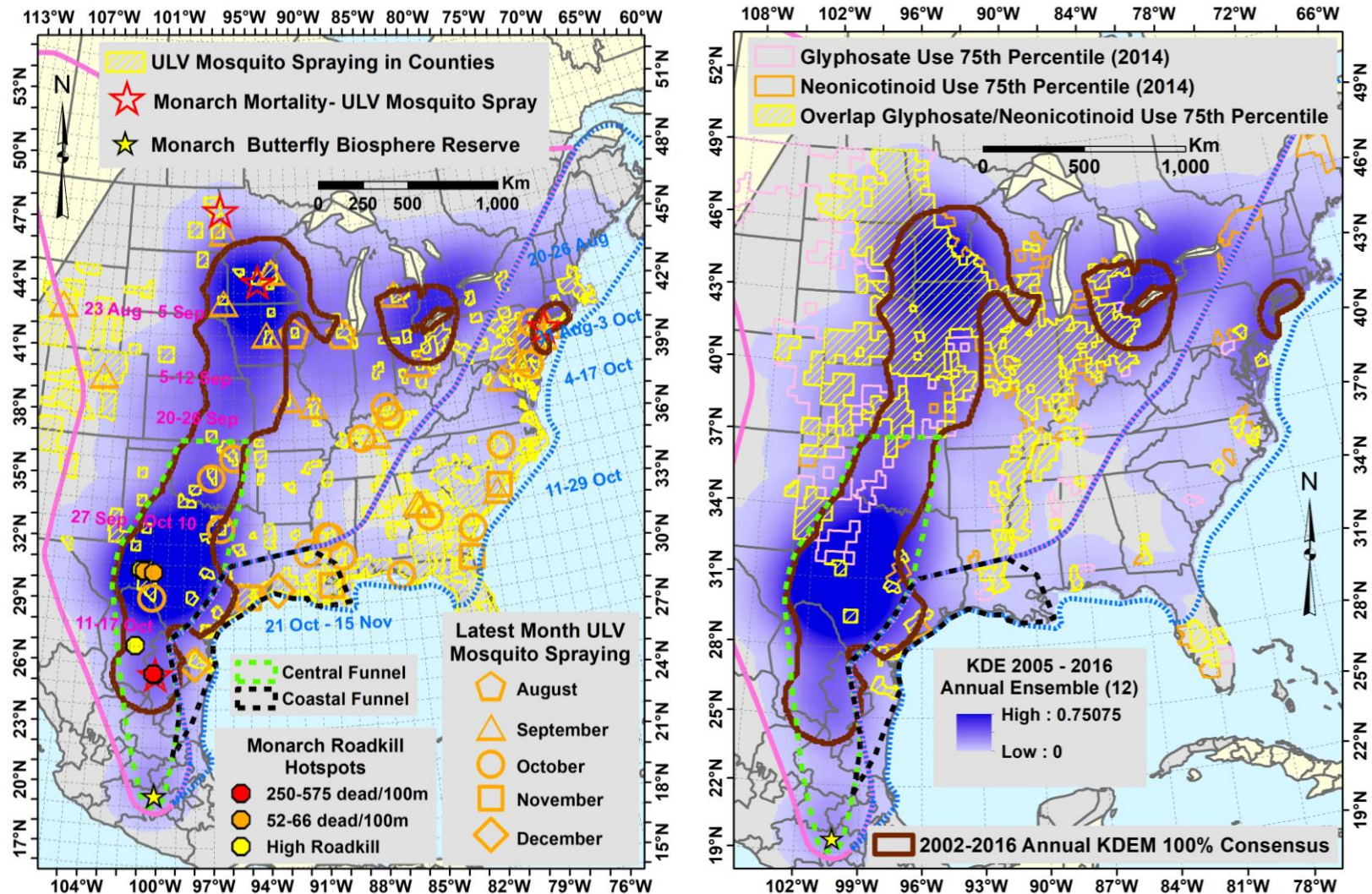


Fig. I.4. Monarch fall migration pathway 100% consensus boundary of annual kernel density estimation models (KDEMs) for 2005-2016 ($n = 12$, blue shading; see Fig. I.3 for individual annual KDEMs) and potential anthropogenic hazards: (A) monarch roadkill hotspots (Table B.5, Fig. B.6), monarch mortality from late season mosquito adulticide ultra-low volume (ULV) spray treatments (Table B.6), months for latest ULV spraying (Table B.7), approximate dates for peak migration along the Central Flyway (pink text) and Eastern Flyway (blue text) (Calvert and Wagner 1999; Howard and Davis 2009), and US counties with ULV spraying; (B) US counties with 75th percentile level uses of glyphosate herbicide and neonicotinoid insecticides (clothianidin, imidacloprid, and thiamethoxam) in 2014 (see Table B.4 for shapefiles).

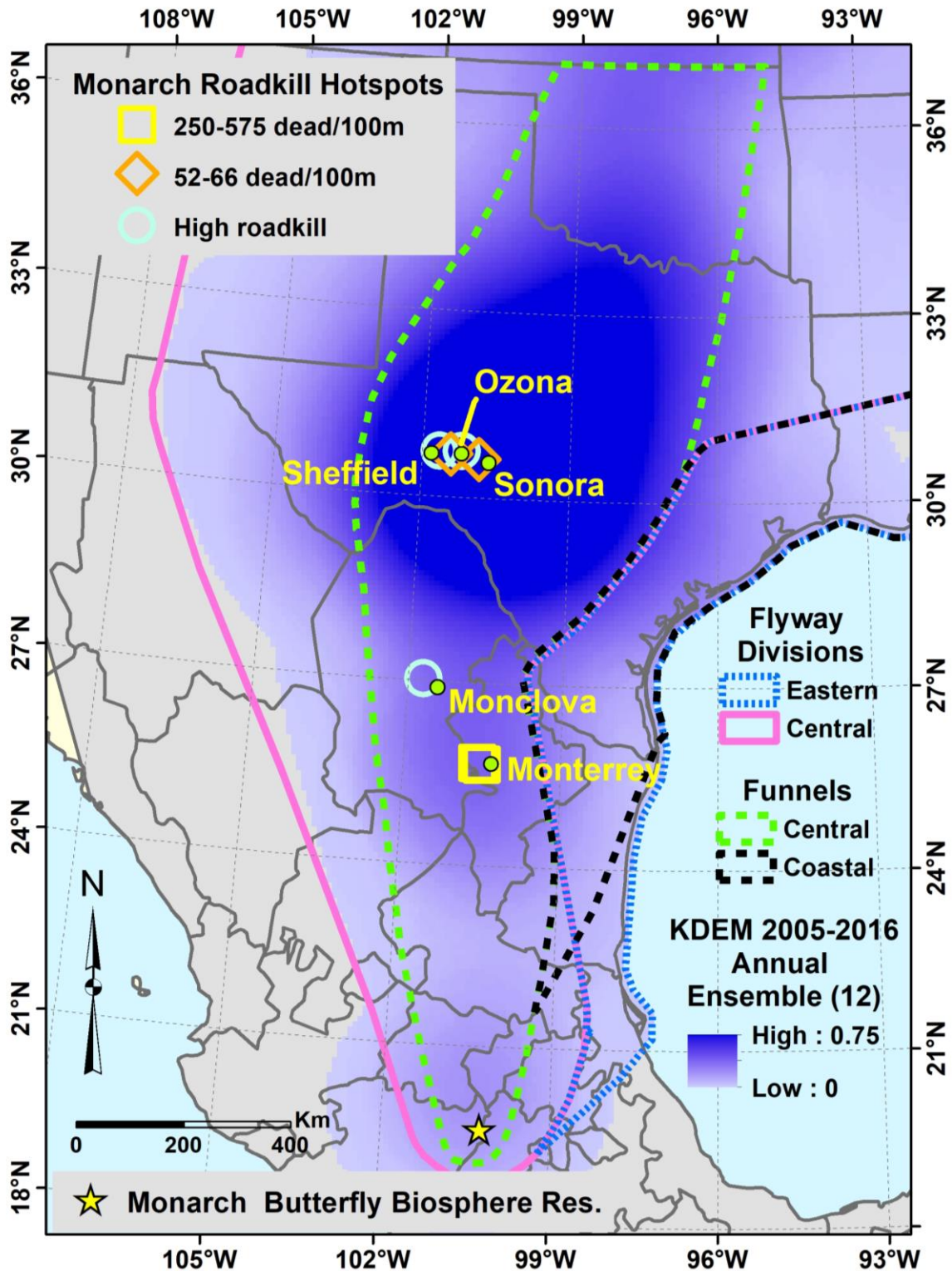


Fig. I.5. Monarch fall migration pathway Central and Coastal funnels and annual kernel density estimation models (KDEMs) for 2005-2016 ($n = 12$, blue shading; see Fig. I.3 for individual annual KDEMs) and monarch roadkill hotspots.

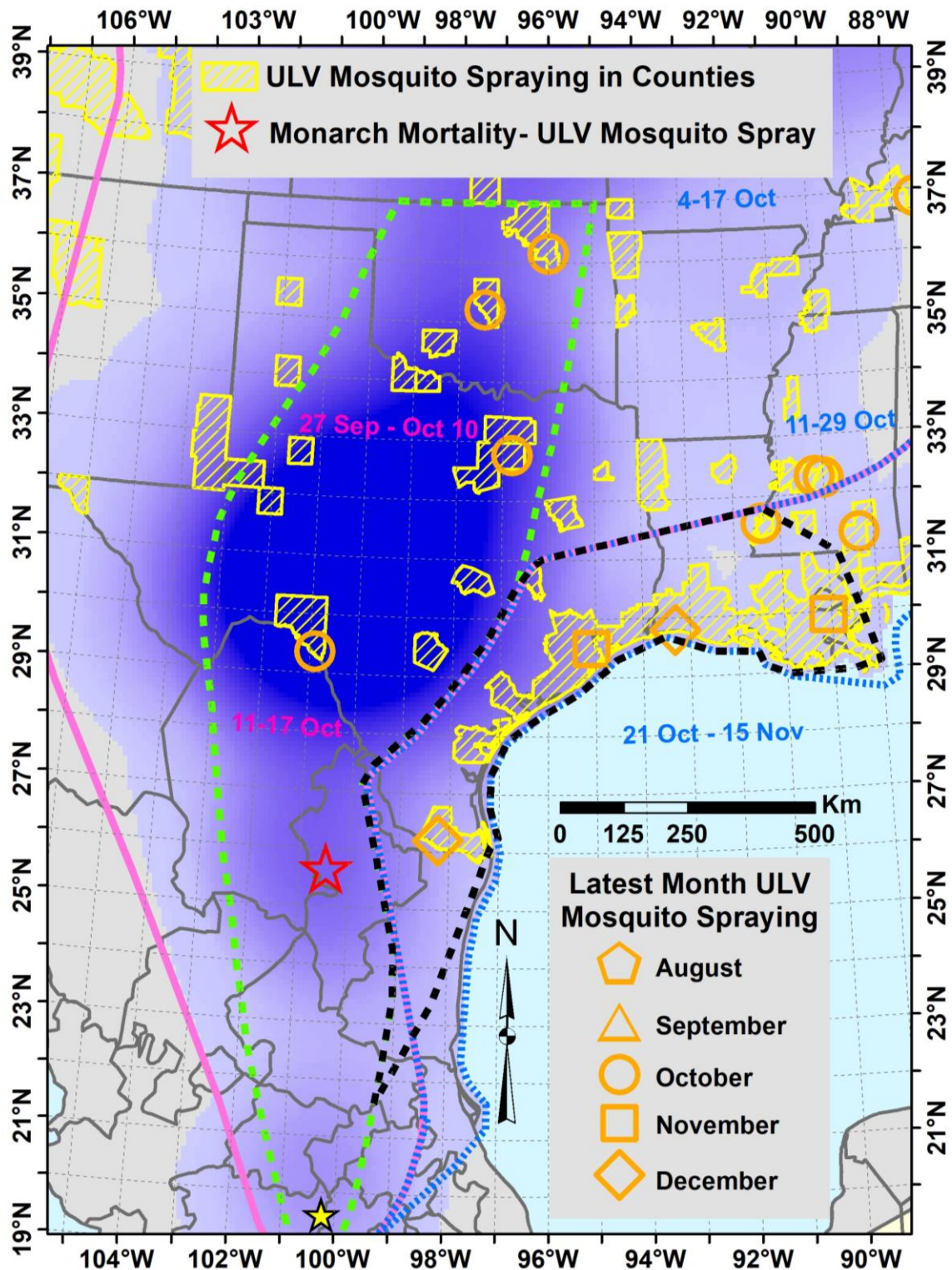


Fig. I.6. Monarch fall migration pathway Central and Coastal funnels and annual kernel density estimation models (KDEMs) for 2005-2016 ($n = 12$, blue shading; see Fig. I.3 for individual annual KDEMs) and US counties with 75th percentile level uses of glyphosate herbicide and neonicotinoid insecticides (clothianidin, imidacloprid, and thiamethoxam) in 2014.

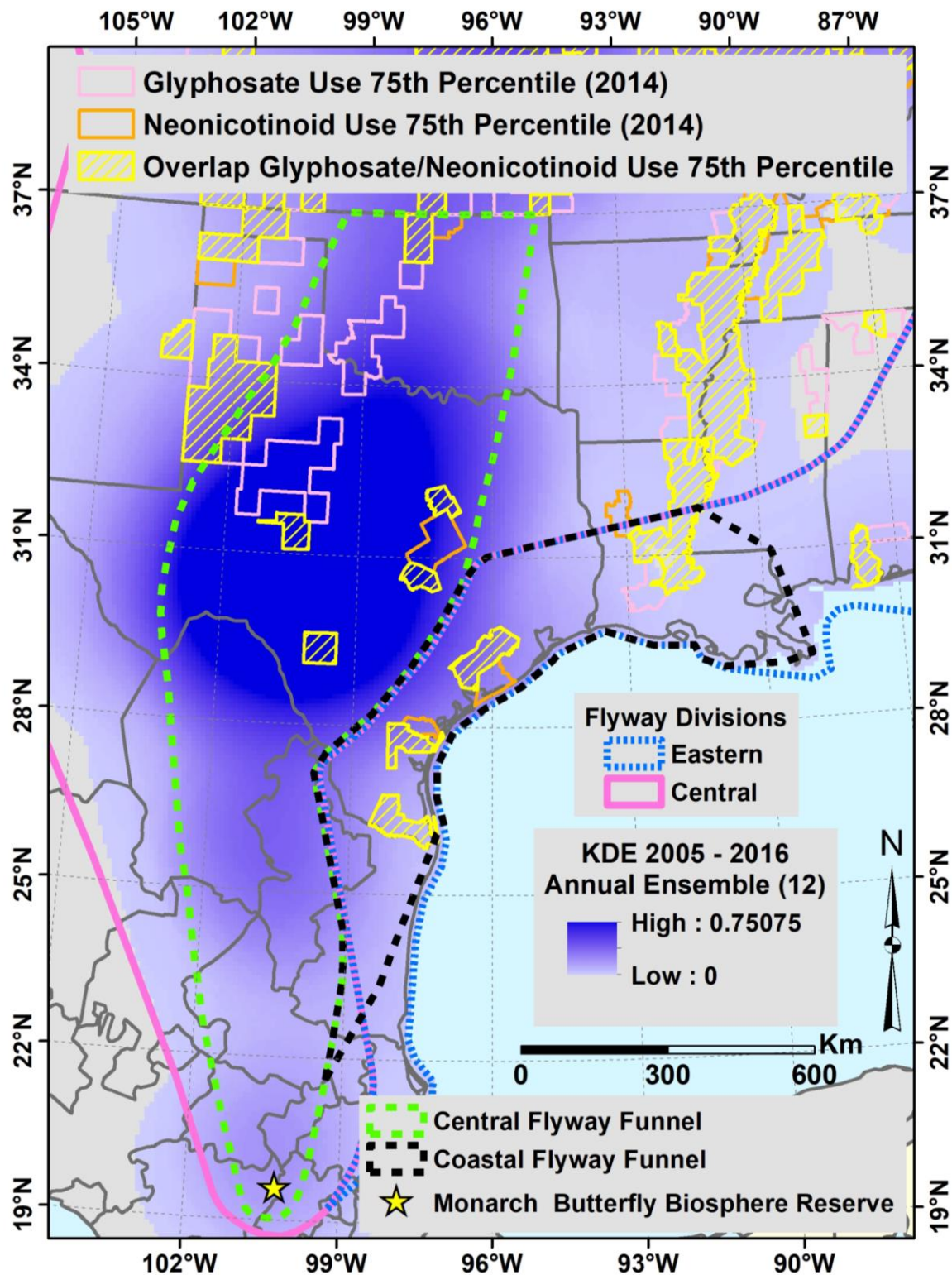


Fig. I.7. Monarch fall migration pathway Central and Coastal funnels with annual kernel density estimation models (KDEMs) for 2005-2016 ($n = 12$, blue shading; see Fig. I.3 for individual annual KDEMs) and US counties with 75th percentile level uses of glyphosate herbicide and neonicotinoid insecticides (clothianidin, imidacloprid, and thiamethoxam) in 2014.

North 2000; permethrin), Grand Forks, North Dakota (Grand Forks Herald 2010; with photo), and Santa Catarina, Monterrey, Nuevo Leon, Mexico (www.Info7.mx 2011; with video) (Fig. I.4A; see Table B.6 for details on dates and numbers of monarch deaths reported). Potential mosquito ULV spray counties comprise about 38% of the portions of Louisiana and Texas within the Coastal Funnel (Fig. I.6). In contrast, only 9-10% of the states with mosquito ULV spraying in the Central Flyway are comprised of potential mosquito ULV spray counties (Figs. I.4A, I.6, Table B.8).

An overlap of the upper 75th percentile usage of glyphosate herbicide and neonicotinoid insecticides was found in US counties for 19 states. Over 30% of the area of these states within the northern Central Flyway had high glyphosate/neonicotinoid usage, including over 75% of Iowa and Illinois, and over 50% of Indiana, Nebraska and North Dakota (Figs. I.4B; Table B.8). In comparison, corresponding percentages of high glyphosate/neonicotinoid counties for the other flyway regions are 11% for the southern Central Flyway (Fig. I.7), 6% for the southern Eastern Flyway, and 1% for the northern Eastern Flyway (Fig. I.4B, Table B.8). The greatest concentration of high glyphosate/neonicotinoid use in Texas is located in the panhandle, mostly to the west of the Central Funnel. In about 40% of the years 2002-2016 (5 of 16 years), almost the entire Texas panhandle fell within the annual KDEM migration pathway (Figs. I.3, I.7). High neonicotinoid use in Texas mostly occurs in the northwestern Central Funnel and panhandle areas (for additional details, see Appendices A, B).

Discussion

The two fundamentally different approaches of interpolative spatial point pattern KDEMs and correlative ecological niche models, such as MaxEnt, provided valuable complementary perspectives on monarch migratory pathways. The various KDEMs were best suited in defining the entire fall migration pathway, and revealing core migratory pathways. The MaxEnt model best revealed the alignment of the migration pathway of the Central Flyway with nectar resources in the Great Plains grasslands. The overlap of the identified core monarch migration pathways with potential anthropogenic hazards of roadkill, mosquito adulticide ULV spray exposure, and the loss and contamination of nectar resources due to agricultural pesticides can help focus future conservation activities and risk assessments.

The Central and Coastal funnels should be the focus for further investigation of potential impacts and mitigation for fall monarch roadkill. Coastal portions of the Eastern Flyway would most benefit from wildflower plantings (e.g., Venturini et al. 2017), perhaps in association with roosting areas and away from roads to mitigate potential negative impacts from both mosquito truck ULV spraying and roadkill. In Texas, intensely cropped regions of the northwestern Central Funnel and panhandle regions are the most important for studying potential benefits of providing additional nectar resources that are also free of pesticide contamination. The MaxEnt model also supports the importance of conserving nectar resources found in grassland habitats of the Great Plains, including throughout the Central Funnel of Oklahoma and Texas. Brower et al. (2015) suggested that nectar sources of Texas and North Mexico are especially crucial for the fall migration (also see Inamine et al. 2016). The tendency of the Central Funnel to shift as much as 200 km in some years, especially towards the West, has important implications for where conservation efforts to support the fall migration should be focused. The KDEM migratory

pathways for about 40% of the annual monarch migrations are shifted far enough west to include most of the Texas panhandle, indicating the importance of this area, even though it does not all fall within the Central Funnel main migratory pathway representative of all years. Spatial risk assessment is needed to determine the degree of exposure to identified potential anthropogenic hazards along the monarch core fall migration pathways. The proposed mitigation strategies for identified threats along the core pathways can provide regional guidance for developing future conservation plans and risk assessments in the continued effort to preserve the unique phenomenon of the eastern monarch fall migration (embedded zipped shapefiles of core migration pathways and potential hazards are provided in Table B.4).

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SECTION II: MONARCH FALL ROADKILL MODEL AND RISK ASSESSMENT

Introduction

Wildlife-vehicle collision is the most widely acknowledged impact of roads on wildlife, and can contribute to the decline of species of conservation concern (Tok et al. 2011, Visintin et al. 2016, Bennet 2017), including globally declining pollinator insect species (Baxter-Gilbert et al. 2015). Migratory danaine butterflies (Nymphalidae: Danainae) may be especially susceptible to roadkill during migration (Her 2008, Taiwan Environmental Protection Administration [EPA] 2010, Santhosh and Basavarajappa 2014), including the monarch butterfly (*Danaus plexippus* L.) in the United States (McKenna et al. 2001) and Mexico (Correo Real 2015).

Road mortality may significantly affect monarch survival during migration, especially where monarchs become highly concentrated as the migration narrows in Texas and northern Mexico (Badgett and Davis 2015). Only McKenna et al. (2001) have previously evaluated monarch butterfly roadkill. They reported monarchs as the second-most killed butterfly species during six weeks of autumn in Illinois. They estimated that more than 500,000 monarchs were killed statewide along interstate highways during one week in early September 1999 (McKenna et al. 2001). There are several unpublished citizen-science reports of locally high monarch roadkill occurrence in West Texas and northern Mexico during the autumn migration, with observed roadkill reaching 5.7 monarchs per meter near Monterrey, Mexico (Correo Real 2015, Journey North 2017). Incidences of high monarch road mortality in northern Mexico have led to the placement of road signs along portions of highways in to reduce speed in the presence of monarchs (Vanguardia 2016).

The extent of monarch roadkill needs to be assessed to estimate its potential contribution to the population decline and support conservation planning. Our goal was to develop MaxEnt niche models for monarch road mortality during the autumn migration within the main migration pathway in Texas. Species distribution modeling has previously been used to project roadkill risk of mammals (Grilo et al. 2009, Roger and Ramp 2009, Visintin et al. 2016, 2017) and owls (Gomes et al. 2009), but spatial models for insect roadkill have not yet been developed. The monarch roadkill models were also projected throughout the Central Funnel, which is the identified main southern autumn migratory pathway within the Central Flyway for monarchs from Oklahoma to Mexico (Tracy 2018, Tracy et al. 2018a). This study includes the first analysis of monarch roadkill data outside of Illinois, and the first development of a spatial roadkill model for an insect. Our specific objectives were to (1) conduct monarch roadkill field surveys within the Central Funnel in Texas, (2) develop MaxEnt niche models for roadkill within the Texas survey area, and project these models throughout the Central Funnel, (3) estimate monarch roadkill numbers within the survey area and the Central Funnel using both simple field survey-based and model-based extrapolation techniques, and (4) discuss the results in the context of monarch conservation and potential applications to other species of conservation concern.

Methods

Monarch roadkill field surveys were conducted within the main autumn migration through the Central Funnel in Texas (Fig. III.1). Four four-day surveys were conducted in each of the

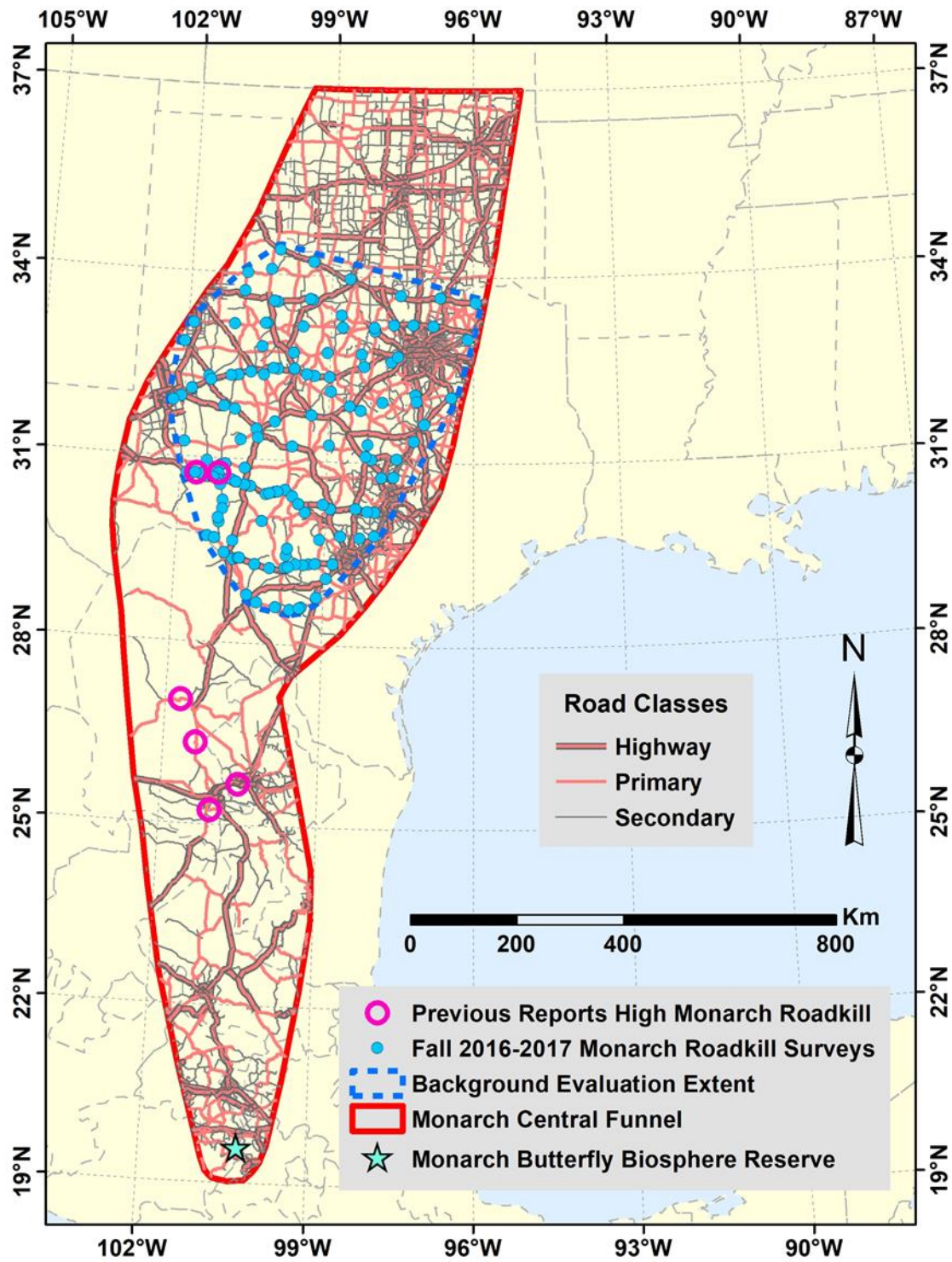


Fig. III.1. Monarch roadkill survey 100 m transects for autumn 2016 and 2017 along three major road classes within the monarch Central Funnel in Texas.

autumns of 2016 and 2017, between 10th October to 4th November and 3-27 October, respectively. The survey area was divided into four north to south sections, with surveys timed to generally occur after the dates of average peak migration (Journey North 2017, Monarch Watch 2018a) to allow time for the accumulation of road-killed monarchs. Surveyed road types included (1) highways, (2) primary roads, and (3) secondary roads. Each survey location comprised at least a single 100 m by one m transect along the grassy edge of one side of the roadway. To assess if the side of the road surveyed influenced the number of dead monarchs, additional transects were surveyed across multiple edges of single and divided-lane highways at some sites. Transects were located using a handheld GPS device (accuracy up to ± 3 m). Roadkill transects were spaced according to travel and survey time constraints at about 30 to 100 km intervals along the primarily east to west pre-planned survey routes, with additional surveys in 2016 in the southwestern portion of the study area where high monarch roadkill was found (Fig. III.1). All dead monarchs or parts of monarchs were collected to estimate the total number and sex ratio of dead monarchs along transects. A similar spring monarch roadkill survey was conducted in Texas during April to May of 2017 (Fig. D.1; for details, see Appendix D, section 1.1). The boundary of the background evaluation extent for our roadkill study was defined by a 10 km buffer around a convex hull polygon formed using un-thinned 2016 to 2017 monarch roadkill survey data (Fig. III.1). We extrapolated the mean roadkill counts for the three road types over the background evaluation extent and Central Funnel in a manner similar to that of McKenna et al. (2001). Roadkill rates in areas of locally high monarch roadkill (hotspots) can vary greatly between years and highly differ from other areas. Consequently, roadkill rates in hotspots can bias roadkill rates in larger areas if they are disproportionally represented in the sampling. Therefore, in some extrapolations, hotspot roadkill rates were considered separately for each year from non-hotspot locations. This separation allowed us to understand the importance of the roadkill hotspots within a year, and compare the hotspot differences between years. We also made roadkill extrapolations including hotspot roadkill rates with non-hotspot data and thinning the hotspot data in 2016 to be in proportion to the sample effort in non-hotspot locations (for details, see Appendix D, section 2.1).

Thirty environmental variables were initially screened for use in the roadkill modeling (Table D.1, Fig. D.2). All indices were either calculated at 30.8 m spatial resolution or resampled with bilinear interpolation to the 30.8 m resolution, to match the resolution of the base layer of one arc second Shuttle Radar Topography Mission (SRTM) digital elevation model (DEM) data obtained from USGS Earth Explorer (<https://earthexplorer.usgs.gov/>). In order to improve model performance, we combined 2016 and 2017 roadkill presence data, which were randomly spatially thinned to 2 km to reduce spatial autocorrelation. Ten thousand background points were randomly generated within the road mask evaluation area. Final MaxEnt roadkill models were developed from ten random sets of ten of the 20 variables to represent model variability due to variable selection. The absolute Spearman rank correlation of variables within random variable sets was limited to less than 0.7 using the multiple randomized sequential forward selection procedure within the random subset feature selection algorithm (RSFSA) of Tracy et al. (2018b). The final MaxEnt models were calibrated to binary presence/absence format using a threshold of maximum TSS_{pa} (Liu et al. 2013) and combined using frequency consensus to form a feature subset ensemble (for additional details, see Appendix C, Kantola et al. 2018 and Appendix D, section 7).

Results

We surveyed 16.1 km of roadsides (161 100 m transects), 8.8 km in 2016 and 7.3 km in 2017. We found 581 dead monarchs in 59 locations (102 absence locations) for an average of 3.4 monarchs per transect. We found 546 dead monarchs in 2016 and 35 in 2017 (for raw survey count data, see Appendix D, section 3.1). Of the 546 monarchs in 2016, 499 (91%) were located along or near IH-10 between Sonora and Sheffield (23 of 95 transects) (Fig. III.2). This area was defined as a single large 2016 hotspot area extending 95 km along Interstate Highway (IH) 10 from Sonora to 24 km east of the Pecos River. This hotspot included a portion of Texas state highway (SH) 163 extending from 7.6 km south of Ozona to 5 km north of Ozona and a 2 km section of SH-137 extending west from the junction with SH-163. The hotspot was bounded along IH-10 in the west and east by counts of 15 and 10 roadkill per 100 m, and included counts of 21 and 6 roadkill per 100 m on SH-137 and SH-163, respectively. About 93% (466/499) of the dead monarchs in the 2016 hotspot occurred in 14 transects ranging from ten to 66 per 100 m (Fig. D.4). A total of 257 monarchs were sexed in 2016-2017, of which ca. 38% were female (n=98). The portion of females was 41% in 2016 (132 males, 91 females) and 21% in 2017 (27 males, 7 females). After the 2 km spatial thinning for spatial modeling, the field survey data consisted of 151 transects (53 presence and 98 absence) and 249 individual dead monarchs. Only two road-killed male monarchs were found among 54 transects in our spring 2017 roadkill survey (Fig. D.1).

Southern edges of roadsides contained 43.8% (\pm 36% SD, n = 13) of the roadkill found in the northernmost edge for both single and divided-lane roads. This relationship was used to estimate roadkill across all two to four edges per roadway when extrapolating roadkill per km per year (for details, see Appendix D, section 5). Estimated roadkill per km ranged from 6.15 to 645 monarchs per km depending on year, road type, and location in relation to the hotspot area (Table III.1). In 2016, mean estimated roadkill per km along highways was significantly higher within the Sonora-Sheffield hotspot (645.38; SD = 446.92; n = 15) compared to outside the hotspot (12.64; SD = 22.30; n = 22) (P = 0.00008; Welch t-test). Between years, the estimated roadkill per km generally varied widely among road types, and there was no evidence of any hotspot in 2017 (Table III.1). Estimates of roadkill within the hotspot area in 2016 and 2017 ranged from about 74,000 to 5,000, representing about 10% and 1 % of the entire mortality within the Texas background evaluation extent. Using the field survey data from the whole area, including the randomly thinned hotspot data, the estimated roadkill for the Central Funnel in 2016 rose to over 3.5 million monarch butterflies (Table III.1). The estimate for 2017 was 1.1 million road-killed monarchs. Combining the data from both of the years yielded an annual estimate of 2.1 million road-killed monarchs. When the hotspot data were separated from the survey data, total estimated annual roadkill rates in 2016 and 2017 were about 1.6 million and 1.0 million monarchs, respectively. In 2016, about 47%-49% of the roadkill within the Central Funnel was projected to occur within the Texas background evaluation extent, of which 5% was projected to occur in the Sonora-Sheffield hotspot area. In 2017, only about 0.3% of the roadkill within the Central Funnel was projected within the hotspot area, and the percent roadkill in the background area ranged from 31% when hotspot data were analyzed separately to 48% when data from the hotspot area were included (Table III.1, Appendix D, section 2).

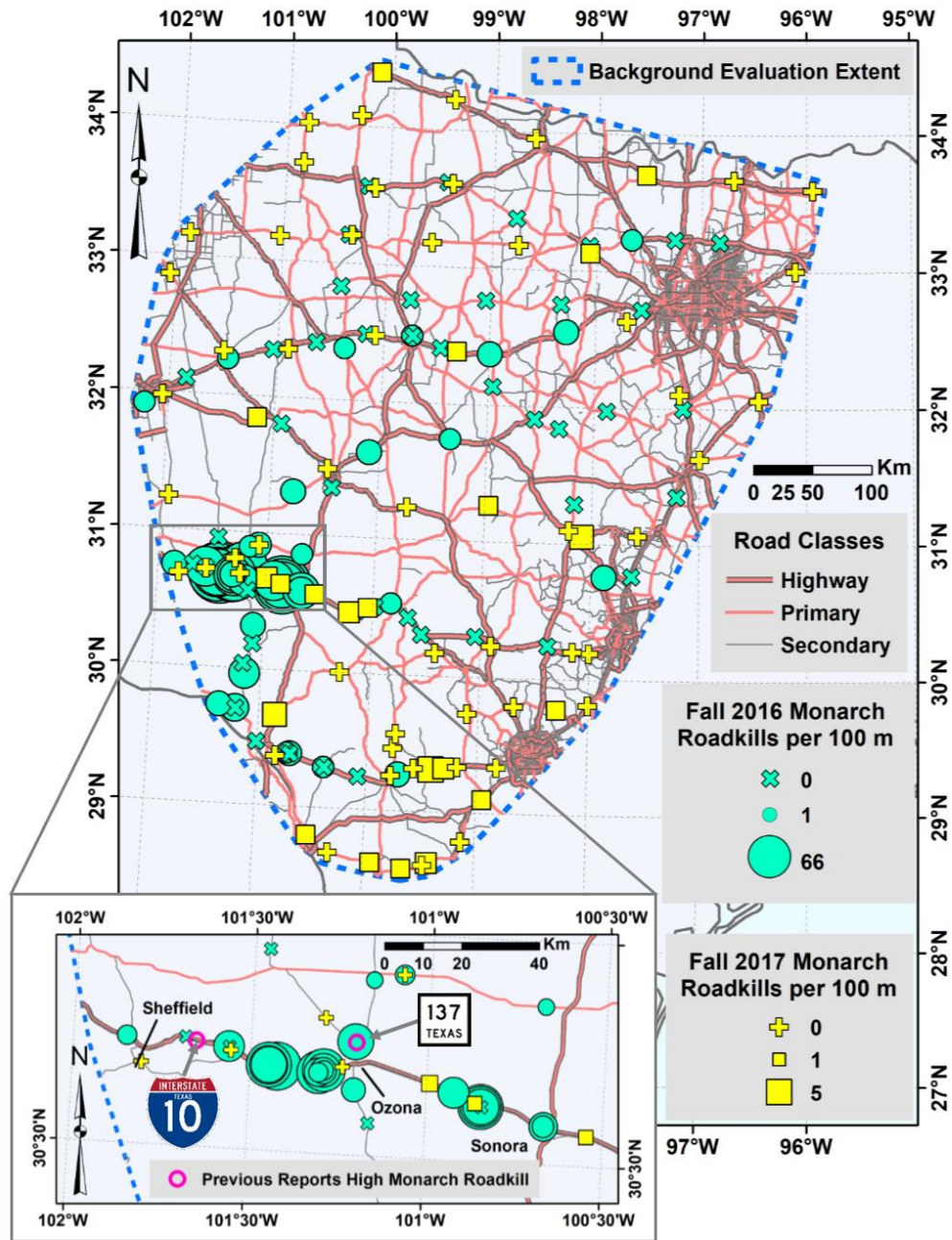


Fig. III.2. Monarch roadkill survey 100 m transects for autumn 2016 and 2017 along three major road classes within the monarch Central Funnel in Texas.

Table III.1. Monarch roadkill estimates per year for 2016 to 2017 for the Sonora-Sheffield roadkill hotspot, Texas background evaluation extent (BEE) and the Central Funnel (Figs. III.8-9) from field data by road type using simple extrapolation or MaxEnt model projections.

Year	Millions of Monarch Roadkill ^a						
	Simple Extrapolation					MaxEnt Model Extrapolations $\bar{X} \pm SD$ (n = 10) ^b	
	Hotspot Data Separated			Hotspot Data Included		Hotspot Data Included	
	Sonora-Sheffield Hotspot	Texas BEE	Central Funnel	Texas BEE	Central Funnel	Texas BEE	Central Funnel
2016							
Millions of Dead	0.074	0.768	1.570	1.663	3.554	2.050 \pm 0.760	3.041 \pm 0.743
% Overwintering Population ^c	0.09%	0.90%	1.82%	1.93%	4.03%	2.36 \pm 0.86%	3.46 \pm 0.82%
% Texas BEE Mortality	9.58%						
% Central Funnel Mortality	4.69%	48.95%		46.78%		66.48 \pm 14.79%	
2017							
Millions of Dead	0.005	0.492	1.042	0.533	1.131	0.738 \pm 0.272	1.084 \pm 0.258
% Overwintering Population ^c	0.01%	0.79%	1.67%	0.86%	1.81%	1.19 \pm 0.43%	1.73 \pm 0.41%
% Texas BEE Mortality	1.02%						
% Central Funnel Mortality	0.32%	31.34%		47.16%		68.86 \pm 14.18%	
2016-2017							
Millions of Dead	0.058	0.561	1.138	0.995	2.118	1.460 \pm 0.540	2.119 \pm 0.510
% Overwintering Population ^c	0.09%	0.91%	1.82%	1.59%	3.33%	1.96 \pm 0.71%	2.82 \pm 0.66%
% Texas BEE Mortality	10.25%						
% Central Funnel Mortality	3.66%	35.75%		27.99%		67.90 \pm 15.08%	

^aMean roadkill rates (roadkill/km/year) (Table D.2) by road type for extrapolations are calculated from transects in the Texas BEE and incorporate estimates for all road edges. Roadkill rates are multiplied by length of road in various areas to obtain extrapolations (see Table D.3 for road lengths used in simple road type extrapolation) (for all calculations, see Appendix D, section 5).

^bSee Table D.4 for individual MaxEnt model estimates.

^cBased on 84.61 (2016) and 61.4 (2017) million overwintering monarchs (Monarch Watch 2018b). Estimated from 21 million monarchs per hectares overwintering (Thogmartin et al. 2017).

The feature subset ensemble of ten MaxEnt roadkill niche models provided excellent discrimination of roadkill presence locations from background locations within the background evaluation extent, with AUC_{bgp} values ranging from 0.82 to 0.88 (0.86 ± 0.02 , mean \pm SD). The models provided marginally poor discrimination of roadkill presences from absences observed in the field, with AUC_{pa} values ranging from 0.60 to 0.67 (0.64 ± 0.02 , mean \pm SD). Among the top eight variables with the highest permutation importance in the MaxEnt models (Table III.2) were three human population density indices and two climatic indices. Other top ranked variables included elevation (m), road density over a three km radius ($km/\sim 28 km^2$), and percent cover of artificial surfaces within a 500 m radius. Traffic volume (annual average daily traffic, AADT) ranked third in permutation importance in preliminary models but was not available for Mexico, precluding its use in our final models.

The highest number of models projected roadkill within much of southwest Texas and Mexico in the Central Funnel (Fig. III.3). None of the MaxEnt models projected monarch roadkill over most of the northeastern Central Funnel, including the Dallas/Fort Worth metroplex and most of eastern Oklahoma. Roadkill extrapolations for each of the ten individual MaxEnt models were made across each year and both years combined. Extrapolations were based upon roadkill rates per km for presence-only transects (no zero roadkill data) of each road type that were multiplied by the MaxEnt model predicted lengths of roadkill presence for the road type. Roadkill rate calculations for extrapolations included data from hotspot transects that were thinned for 2016 as done for simple extrapolations (for details, see Appendix D, sections 2.1, 5). Extrapolating roadkill rates across the Central Funnel, we estimated averages of 3.0 ± 0.7 and 1.1 ± 0.3 million (mean \pm SD) road-killed monarchs for 2016 and 2017, respectively (Tables III.1, D.2). Combining roadkill data across both years for the Central Funnel yielded annual roadkill rates of 2.1 ± 0.5 million. The mean projected percentage of road-killed monarchs in the Central Funnel that occurred within the Texas background evaluation extent ranged from 67% to 68% in 2016 and 2017 (Tables III.1, D.4).

Discussion

In our 2016 roadkill survey (but not 2017), we found a Sonora-Sheffield, Texas, monarch roadkill hotspot that corresponds to the only two previous citizen-science reports of monarch roadkill hotspots in the US (Fig. III.2). It is unclear why hotspots have repeatedly occurred in this area. The hotspot location may be partly related to higher densities of migrating monarchs in more southern areas of the Central Funnel.

The observed variation in roadkill rates falls within previous reports for monarchs of from 1.3 to 11.9 butterflies per km per week in Illinois (McKenna et al. 2001) to a very high number of 115 road-killed monarchs within a 20 m stretch along toll highway 40D southwest of Monterrey, Mexico in October 2015 (Correo Real 2015; see Appendix D, section 8 for data). Reported roadkill rates for other butterflies have ranged from 0.45 to 80 per km per day in North America, Asia, and Europe (Rao and Girish 2007, De la Puente et al. 2008, Yamada et al. 2010, Skórka et al. 2013, Baxter-Gilbert et al. 2015). The observed sex ratios of roadkill monarchs were also consistent with previous studies. Several factors contribute to the conservative nature of our estimates, including the difficulty in detection of dead monarchs, especially in taller

Table III.2. MaxEnt model variable permutation importance for 19 variables used in ten random sets of ten of the 20 variables in monarch roadkill models.

Variable ^a	Abbreviation	Permutation Importance ^b , Mean \pm SD
Human population density per km ² in 9 km radius (population/254.5 km ²)	<i>popden9kr</i>	44.2 \pm 3.8 (3)
Autumn quarterly mean monthly actual evapotranspiration/potential evapotranspiration \times 1000	<i>etrt_autq</i>	41.7 \pm 23.3 (2)
Annual mean monthly rainfall (mm)	<i>prec_ann</i>	36.4 \pm 15.6 (4)
Human population density per km ² in 3 km radius (population/28 km ²)	<i>popden3kr</i>	30.1 \pm 0.0 (1)
Elevation (m)	<i>elev</i>	27.9 \pm 5.5 (4)
Distance to urban areas (areas with \geq 300 humans per km ²) (km)	<i>urbdist</i>	27.1 \pm 10.8 (4)
Road density, km road in 3 km radius (km/28 km ²)	<i>roadden3kr</i>	19.6 \pm 2.4 (2)
Artificial surfaces % cover in 500 m radius	<i>artsur_500mr</i>	13.4 \pm 3.2 (3)
Autumn quarterly mean monthly maximum temperature ($^{\circ}$ Celsius)	<i>tmax_autq</i>	10.5 \pm 9.8 (3)
Distance to highways (m)	<i>hwydist</i>	10.4 \pm 2.5 (7)
Latitude (decimal degrees)	<i>latitude</i>	6.4 \pm 4.3 (3)
Grasslands % cover in 500 m radius	<i>grslnd_500mr</i>	5.6 \pm 3.4 (9)
Autumn mean quarterly wind speed (m/second)	<i>wndsp_autq</i>	5.6 \pm 5.4 (4)
Distance to secondary roads (m)	<i>secrddist</i>	5.1 \pm 2.9 (8)
Shrublands % cover in 500 m radius	<i>shrub_500mr</i>	4.5 \pm 3.4 (9)
Distance to primary roads (m)	<i>primrddist</i>	2.3 \pm 3.0 (4)
Road density, km road in 500 m radius (km/0.78 km ²)	<i>roadden500mr</i>	2.2 \pm 3.8 (5)
Distance to High Flow Accumulation Areas ($>60,000$ ~km ² upslope grid cells) (m)	<i>strmhiflodist</i>	1.2 \pm 1.0 (5)
Topographic Position Index (TPI), 3 km radius	<i>tpi3kr</i>	1.0 \pm 0.2 (5)
Cultivated land % cover in 500 m radius ^c	<i>cult_500mr</i>	0.0 \pm 0.0 (0)

^a See Table D.1 for sources of variables. Variables ordered from highest to lowest mean permutation importance.

^b Permutation importance of the variable in the MaxEnt models, number of models in which the variable was used out of the 10 random models in parentheses.

^c Cultivated land layer was initially included in all 10 random models, but it was not included by MaxEnt in calculating any of the models.

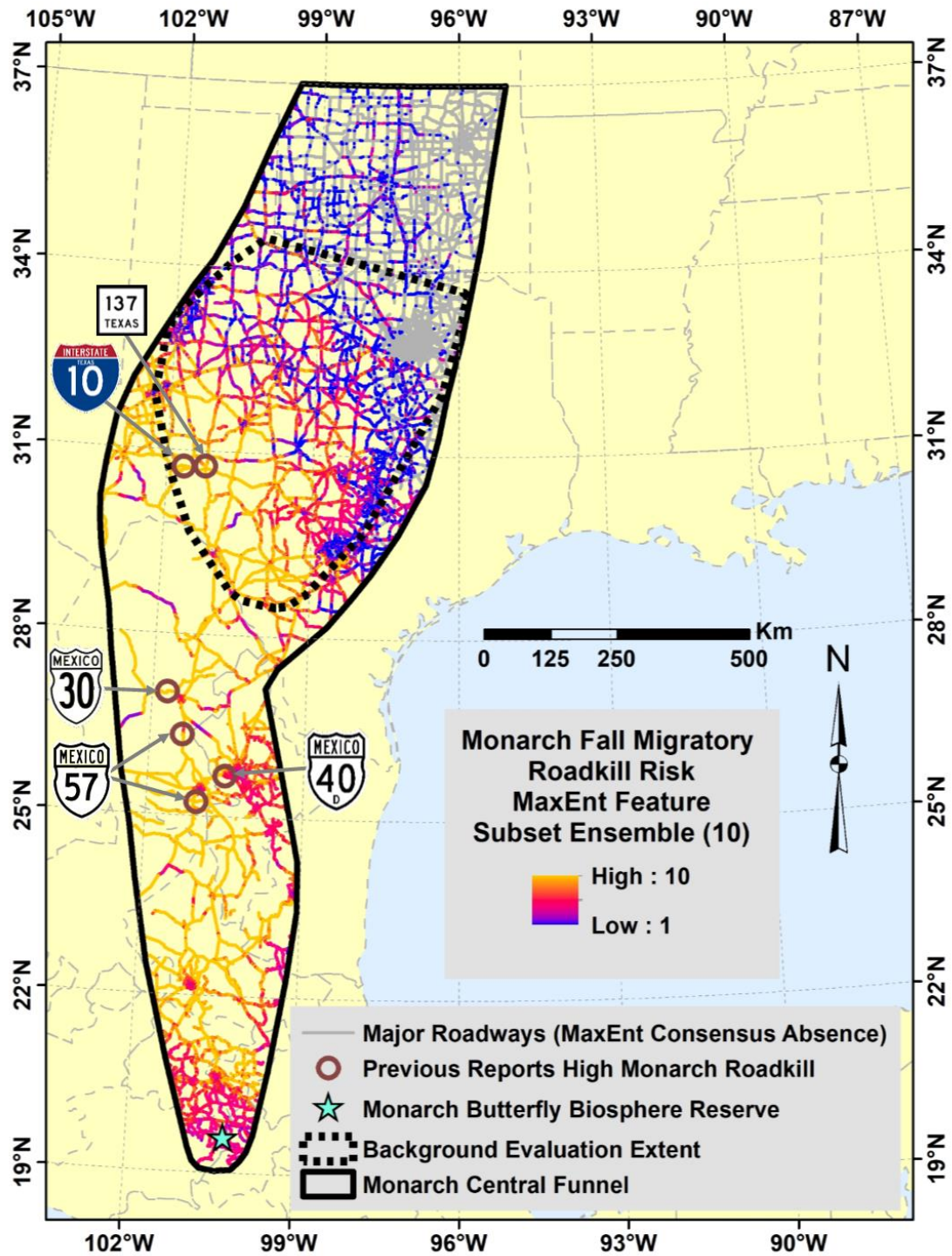


Fig. III.3. MaxEnt frequency consensus for feature subset ensemble of ten models developed from random subsets of ten of 20 variables correlated less than 0.7 (see Appendix D section 6 for embedded zipped shapefile of MaxEnt consensus model).

vegetation, shredding of dead monarchs by roadside mowing, and a portion of the monarchs remaining attached to the colliding vehicles (McKenna et al. 2001, Seiler and Helldin 2006). However, several studies indicate that road killed butterflies are mostly blown to the roadside edge, with individuals rarely trapped in car grills (Munguira and Thomas 1992, Ries et al. 2001). The overall agreement of our roadkill results with previous studies in terms of the range of roadkill rate variability and sex ratios supports the reliability of the data for extrapolating monarch road mortality according to road types and spatial modeling.

Most of the MaxEnt models projected monarch roadkill from the southwestern portion of the Central Funnel from West Texas to Mexico (Fig. III.3). Our MaxEnt consensus projection agrees with all seven of the previously known citizen science reports of monarch roadkill hotspots in North America (Fig. III.3), including two hotspots in West Texas (Journey North 2017) and five hotspots in northern Mexico (Correo Real, 2015; Rogelio Carrerra, Universidad Autonoma de Nuevo Leon, Nuevo Leon, Mexico, personal communication).

Our annual MaxEnt based roadkill estimates for monarch mortality throughout the Central Funnel were 3.0 to 1.1 million for 2016 and 2017, respectively. These MaxEnt roadkill estimates were similar to those based on simple roadkill extrapolation by road type when hotspot data were included (3.6 and 1.1 million, respectively). Most of the roadkill projected by MaxEnt models outside of the study area occurred in Mexico, indicating that more MaxEnt models are projecting roadkill along the sparser road network within the Central Funnel over northern and central Mexico than in the northern parts of the funnel (Fig. III.3). As the autumn migration pathway narrows in the South, migrating monarchs become more concentrated in the Central Funnel. This higher concentration may contribute to higher roadkill densities in the southern parts of the Central Funnel in Mexico, where most previous reports of monarch roadkill hotspots originate (Fig. III.3). Although our models project some increased southward mortality risk, additional data are needed to assess the extent of this risk in Mexico. The extrapolations including the hotspot data with other roadkill data and projecting higher annual roadkill of up to 3.6 million in the Central Funnel should be more realistic considering the occurrence of multiple roadkill hotspots in Mexico of higher density than seen in Texas. The MaxEnt models project little to no roadkill in the northeastern part of the funnel area, including the Dallas/Fort Worth area and eastern Oklahoma. However, some roadkill likely does occur in this area, but possibly at a lower rate than in the more southern areas of the Central Funnel. Further research and field surveys are needed to verify roadkill rates outside of our survey area in the southern and northern parts of the Central Funnel. Additional data may allow effective use of roadkill density models, rather than presence only MaxEnt models used in this study. Roadkill density models can better reveal regional roadkill patterns useful in refining projections, especially for northern Mexico where roadkill could be much higher than in Texas. Roadkill field surveys from other parts of the autumn migration pathways could improve estimates for the total impact of roadkill on the monarch population.

MaxEnt projections of monarch roadkill within the Central Funnel were generally associated with more arid climate and less densely populated areas (Appendix C, Fig. 3). These conditions generally describe those for the seven previous monarch roadkill hotspots reported from Texas and Mexico, with the possible exception of the roadkill hotspot in the vicinity of Monterrey, Mexico (Fig. III.3). This could be related to a variety of factors. For example,

autumn migrating monarchs have been observed to spend additional time flying lower to the ground during the afternoon in desert areas, perhaps to seek shelter from the heat or find nectar (Journey North 2018). Monarchs may need to spend more time searching for nectar in arid environments, although this has not been evaluated. Finally, the increased roadkill rates may simply reflect the increased number of monarchs in more southern areas of the Central Funnel. This southern locality factor cannot be associated with most of the predictors, with the exception of latitude, but it may have the highest influence. Local climate, weather patterns, and geography affect monarch movement and behavior, and they all are likely important contributors to road mortality. Wind patterns (direction, duration, and speed) may especially be more important than anthropogenic factors, but short-term weather events could not be incorporated in the models. Occurrence of these weather events is also highly variable and difficult to predict.

In general, higher roadkill has been associated with higher traffic volume due to increased probability of vehicle collisions (Seiler and Helldin 2006, Skórka et al. 2013). Samways (1994) suggested that roads with high traffic volume serve as corridors for high butterfly mortality. In our study, low traffic volume, along with related anthropogenic variables, is correlated with locations of monarch roadkill hotspots, but may not be directly related to the roadkill mortality. As the human population grows, traffic volume should increase, including in the lower traffic volume monarch roadkill hotspot areas, likely leading to higher roadkill rates (Bennet 2017).

Over 70,000 monarch roadkill were estimated in the Sonora to Sheffield, Texas hotspot in 2016, compared to about 5,000 in 2017. This illustrates the high spatio-temporal variability of roadkill and the potential contribution of hotspots to the monarch road mortality. The timing of our field surveys relative to the peak migration in those years may have varied among transect locations and years, which could have influenced our results. If peak migration occurred after our field surveys in one or both years, we may have underestimated roadkill numbers. For example, the autumn migration in 2017 was later than usual due to unusually hot summer (Agrawal and Inamine 2018). Few other studies have identified roadkill hotspots for butterflies (but see Samways 1994, Her 2008). Monarch roadkill hotspots may vary from year to year and may be difficult to locate or may not occur in some years.

We observed much higher monarch road mortality during autumn migration than in the spring (Fig. D.1), indicating that seasonality is a factor contributing to monarch roadkill rates. However, the monarch spring migration is more spatio-temporally dispersed making comparisons difficult. Temporal variability can occur between years, within the migration phase, or even within a day. For example, monarch behavior, such as flying low in the mornings (McKenna et al. 2001), probably affects the risk of roadkill at different times of day. Temporal patterns affecting monarch roadkill should be investigated further. Other sampling methods may be effective in detecting additional roadkill hotspots, such as adaptive cluster sampling, which was developed for inventorying scattered and clustered phenomena (Thompson 1990). More frequent samples in a given location can also be critical for detecting roadkill hotspots (Santos et al. 2015).

Butterfly roadkill is a density independent mortality factor (Rodewald and Gehrt 2014) with the incidence of roadkill being subject to high variability. Consequently, roadkill percentage of the migrating population is very unpredictable in any given year. We estimated a conservative

annual percentage of migrating monarchs subject to road mortality in the Central Funnel by dividing a roadkill estimate by the sum of overwintering populations and roadkill estimate (overwintering cohort). The size of the overwintering populations is calculated by multiplying the hectares of roosting monarchs at the Mexican overwintering sites from Rendón-Salinas et al. (2018) by the value of 21.1 million monarchs per ha suggested by Thogmartin et al. (2017). Based on these amounts, the estimated annual roadkill rates from our combined years of 2016-2017 MaxEnt models in the Central Funnel represent an average of 2.8% (2.1 million) of the overwintering cohort. In 2016, roadkill within the Central Funnel may have represented about 4.0% of the overwintering cohort (Table III.1). The corresponding roadkill for 2017 ranges from 1.5% to 1.8% of the overwintering cohort. In contrast, the projection of 500,000 road-killed monarchs by McKenna et al. (2001) in Illinois during one week in 1999 represents only 0.26% of the overwintering cohort that year (191 million, 9.05 ha), indicating potentially lower roadkill rates for areas north of the Central Funnel in some years.

The actual monarch population decline within a given year can be much higher than the differences in overwintering cohort sizes from year to year, since it includes the unknown quantities of butterflies recruited, or not recruited due to habitat loss, during spring and summer breeding. Mortality at the Mexican overwintering sites due to occasional winter storms can produce very high mortality, estimated at around 75% in 2002 (Brower et al. 2004) and greater than 40% in 2016 (Brower et al. 2017). Average monarch overwintering mortality is around 36% \pm 21% (n = 7; range 4-55%) based on data from Ries et al. (2015; see their Fig. 24.7). Lowered recruitment due to loss of milkweed habitat has been identified as the primary factor in monarch population declines (Pleasants 2017, Thogmartin et al. 2017), with losses of milkweed resources in Iowa estimated at 76% from 1999-2014 (Pleasants et al. 2017). Other studies place more importance on mortality during the autumn migration in the decline of monarchs (Badgett and Davis 2015, Inamine et al. 2016, Agrawal and Inamine 2018). Consequently, it is complex to evaluate the relative importance of roadkill to other factors in the monarch population decline, but it is probably lower compared to the factors of milkweed habitat loss and overwintering mortality. Northward expansion of the monarch summer breeding range is anticipated with climate change (Batalden et al. 2007, Lemoine 2015), making the southward autumn migration route even longer, increasing both exposure to traffic and associated road mortality (Badgett and Davis 2015). Monarch roadkill during autumn migration should be further evaluated in the context of other mortality factors along the migration path (Baxter-Gilbert 2015). A 0.5% annual reduction in migrating monarch mortality through roadkill mitigation could significantly contribute to a reversal in the long-term 7.2% annual exponential decline in monarch populations (see Fig. 1, INTRODUCTION).

While some major factors in the monarch decline are difficult to reduce, such as overwintering mortality, the opportunity for reducing road mortality is possible through roadkill mitigation (see Rytwinski et al. [2016] for a review on mammal roadkill mitigation). The potential ability to locate re-occurring monarch roadkill hotspots could facilitate more effective mitigation. Scattered and unpredictable monarch roadkill would be more difficult to mitigate. A variety of roadkill mitigation measures have been implemented for danaine migratory butterflies. In Taiwan, a four-meter high net was placed along a 400 m section of bridge on National Freeway 3 to successfully induce spring migrating purple crow butterflies (*Euploea* spp.) to fly over and above the traffic, reducing on site roadkill from around 2.5% to 0.5%. In addition, an

outer traffic lane was closed when more than 500 butterflies per minute were crossing (Her 2008, Taiwan EPA 2010). In response to heavy autumn migratory monarch roadkill observed in Coahuila state of Mexico, traffic signs were posted in 2015 limiting the maximum speed to 60 km per hour (37 mph) in the presence of monarchs (Miranda 2015). Police have been observed slowing traffic in Nuevo Leon state in Mexico to reduce monarch mortality along a highway south of Monterrey (Dr. Orley R. Taylor, personal communication). Additional research is needed to test and assess the effectiveness of these types of butterfly roadkill mitigation strategies for monarch roadkill hotspots in west Texas and Mexico.

Our study represents a novel approach for projecting roadkill of a migratory insect through ecological niche modeling. Annual monarch roadkill rates during autumn migration varied substantially. We found close agreement between two methods of estimating monarch roadkill rates, simple extrapolation by road type and MaxEnt roadkill model projections. We project about 1.0 to 3.6 million road-killed monarchs per year during autumn migration over the Central Funnel, which could represent 2-4% of the Mexican monarch overwintering population. MaxEnt model roadkill projections also aligned with several previously known monarch roadkill hotspots, which suggests MaxEnt models could be used to identify additional monarch roadkill within the Central Funnel. Roadkill rates may differ throughout the Central Funnel compared to those observed in the Texas survey area, and should be further investigated. Monarch road mortality should also be investigated along the Coastal Funnel, especially from Texas to Mexico. We recommend more detailed investigation into the spatial and temporal variability in monarch road mortality in the Central Funnel, including how local and short-term weather events, especially related to wind, influence monarch roadkill hotspots. With the new information on monarch road mortality in the Central Funnel, conservation efforts could be implemented to mitigate mortality at monarch roadkill hotspots. Reducing roadkill rate over the Central Funnel for the eastern monarch population is as an important step towards reversing the continuing decline of this iconic butterfly.

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SECTION III: MONARCH MILKWEED HABITAT MODELS AND LAND COVER RISK ASSESSMENT

Introduction

Studies of factors contributing to the decline of the eastern population of migrating monarch butterflies have been primarily focused on the loss of milkweed habitat in the Midwest US for second and third generation larvae (Oberhauser et al. 2017, Pleasants et al. 2017, Thogmartin et al. 2017) and adult mortality within the Central Mexico overwintering grounds (Brower et al. 2004, 2012, 2017; Ries et al. 2015). Few studies have focused on monarch population risks in the South-Central US states of Texas, Louisiana, Arkansas, and Oklahoma (but see Tracy et al. 2019, Kantola et al. 2019). This region is critical for development of monarch spring first generation and the late summer and fall fifth generation larvae (Oberhauser et al. 2017). The primary host plants of monarch larvae are milkweeds of the genus *Asclepias*, of which 33 of the 108 known species in North America are documented as larval host plants (Ackery and Vane-Wright 1984, Malcolm and Brower 1986, Lynch and Martin 1993). Spring breeding in the South-Central US has been reported on *A. oenotheroides* in south Texas (Calvert 1996), *A. amplexicaulis* and *A. viridiflora* in Northeast Texas, *A. viridis* in Texas and western Louisiana, and *A. asperula capricornu* in Texas (originally as *A. asperula* here and in remainder of paragraph, Lynch and Martin 1993). Calvert (1999) reported fall fifth generation larvae in Texas from *A. viridis*, *A. a. capricornu*, *A. oenotheroides*, and *A. latifolia* (in order of abundance). The US Fish and Wildlife Service (USFWS; Tuggle 2014) has identified the first three of these milkweed species, *A. a. capricornu*, *A. viridis*, and *A. oenotheroides*, as the most important for monarchs in Texas, and the USDA Natural Resource Conservation Service (USDA NRCS 2015) has targeted these three species for conservation efforts in their south-central subregion, which includes Texas, Oklahoma, and Kansas. We regard *A. latifolia* along with these three species as the four primary native milkweeds potentially most important as spring and late summer/fall hosts for monarch larvae in the South-Central US. All four of these native milkweed species are herbaceous perennials blooming mostly from spring to summer (Woodson 1954).

Primary threats to native milkweeds are unfavorable land cover/land use change. Significant populations of common milkweed, *A. syriaca*, and associated 2-3 generation monarch larvae, have been lost in recent years due to increased use of glyphosate herbicides among corn and soybean crops in the Midwest (Pleasants 2017). In contrast to *A. syriaca* in the Midwest, which readily grows in cropland with its rhizomatous roots, the above mentioned four South-Central milkweeds are generally stout rooted (Singhurst et al. 2015), and persist poorly in cultivated cropland and haylands. These southern milkweeds are all tall shade intolerant species that are adapted to patchwork disturbance from historical bison grazing and wildfires of the Great Plains (USDA NRCS 2015). With the exception of MaxEnt models developed for *A. viridis* (Lemoine 2015), niche models for the four South-Central US milkweeds are mostly lacking. In comparison to data on *A. syriaca* (Pleasants 2017), information is also lacking on the utilization of different land use/land cover types by the four South-Central milkweeds. The association of fine spatial resolution occurrence data for milkweed species with 30 m resolution National Land Cover Database (NLCD) layers (Multi-Resolution Land Characteristics Consortium [MRLC], 2017) could be used in identifying the most suitable land cover types within core distributions derived from niche models not directly incorporating land cover.

The objectives of this study were to (1) develop range-wide niche models for the four primary native milkweeds of the South-Central US, *A. viridis*, *A. a. capricornu*, *A. oenotheroides*, and *A. latifolia*, and (2) develop a land cover risk assessment over the modeled core niches of each milkweed species for the South-Central US region. For the last objective, we developed a novel land cover utilization index to score the utilization of 2011 NLCD land cover types by each milkweed species.

Methods

Occurrence data throughout the entire North America ranges for the four primary South-Central milkweed species were obtained from a variety of sources, including iNaturalist (2018; also source for milkweed images), SEINet (2017), Global Biodiversity Information Facility (GBIF 2017), vPlants (2017), 2017 correspondence with various herbaria (Kansas State University [KSC], Missouri State University [MSU], Nebraska State University [NSU], and the Oklahoma State University [OSU] Oklahoma Vascular Plants Database), and Texas survey data from our laboratory and various collaborators (Janice Bush, University of Texas at San Antonio, 2017; David Berman, Oklahoma State University, 2017). An effort was made to obtain occurrence data representative of the known county distributions of milkweed species (Kartesz 2015), such as through georeferencing SEINet and vPlants records in various counties from which we lacked specific locations. Some of the herbarium milkweed data (e.g., OSU) also required georeferencing from detailed locality descriptions, such as distances from landmarks along named highways. We compiled additional data on the distribution of *A. asperula capricornu* to the east and *A. a. asperula* to the west by subspecific identification of plants in images from iNaturalist (2018) observations using key taxonomic characters of flower dorsal hood color, flower peduncle length, and the narrowness of leaves (Woodson 1954). This data was used to define the geographic area from which *A. a. capricornu* locations were accepted for niche modeling.

An initial set of 95 environmental variables at 1 km resolution were used in developing MaxEnt niche models, including 57 climatic indices, 14 topographic indices, and 24 soil indices (Table E1). MaxEnt version 3.3.3 models (Phillips et al. 2006) were developed from milkweed locations using the R-software (R Core Team 2017) dismo package (Hijmans et al. 2011). The occurrence locations were thinned by a 10 km spatial filter for reducing sample bias and spatial autocorrelation among the data (Boria et al. 2014). About 10,000 pseudoabsence points were generated for model evaluation within the background evaluation extent, consisting of a 500 km buffer around a convex hull polygon of the occurrence points. The pseudoabsences were buffered at 20 km from the presence points (e.g., Barbet-Massin et al. 2012). The R PresenceAbsence package (Freeman and Moisen 2008) was used with presence and pseudoabsence points for calculating a pseudoabsence (psa) version of the true skill statistic (TSS_{psa}) and area under the curve statistic (AUC_{psa}) using random cross validation. This is in contrast to the standard practice of utilizing the MaxEnt software default calculation of a background presence (bgp) version of AUC (AUC_{bgp}), which incorporates as absences both background and presence points (not buffered pseudoabsence points). To reduce MaxEnt model complexity and overfitting for improved model generalization (Jiménez-Valverde et al. 2008; Warren and Seifert 2011), we adjusted the MaxEnt beta regularization to two and used only quadratic and hinge features (Tracy et al. 2018). We utilized the random subset feature selection

algorithm (RSFSA) to evaluate performance of thousands of MaxEnt models developed from random combinations of subsets of 3 to 25 of the 95 variables, limiting correlation of variables to less than |0.7| (Tracy et al. 2018). The top 12 feature-selected MaxEnt models of six to eight variables each were calibrated to binary presence/absence format using a threshold of maximum TSS_{psa} (Liu et al. 2013). These models were combined using frequency consensus to form an ensemble of models derived from different selected feature subsets (feature subset ensemble). The 100% consensus of the 12 models was defined as the core habitat for the species. Core habitats of each species were identified with portions of Level II and III ecoregions of the Commission for Environmental Cooperation (CEC 2005).

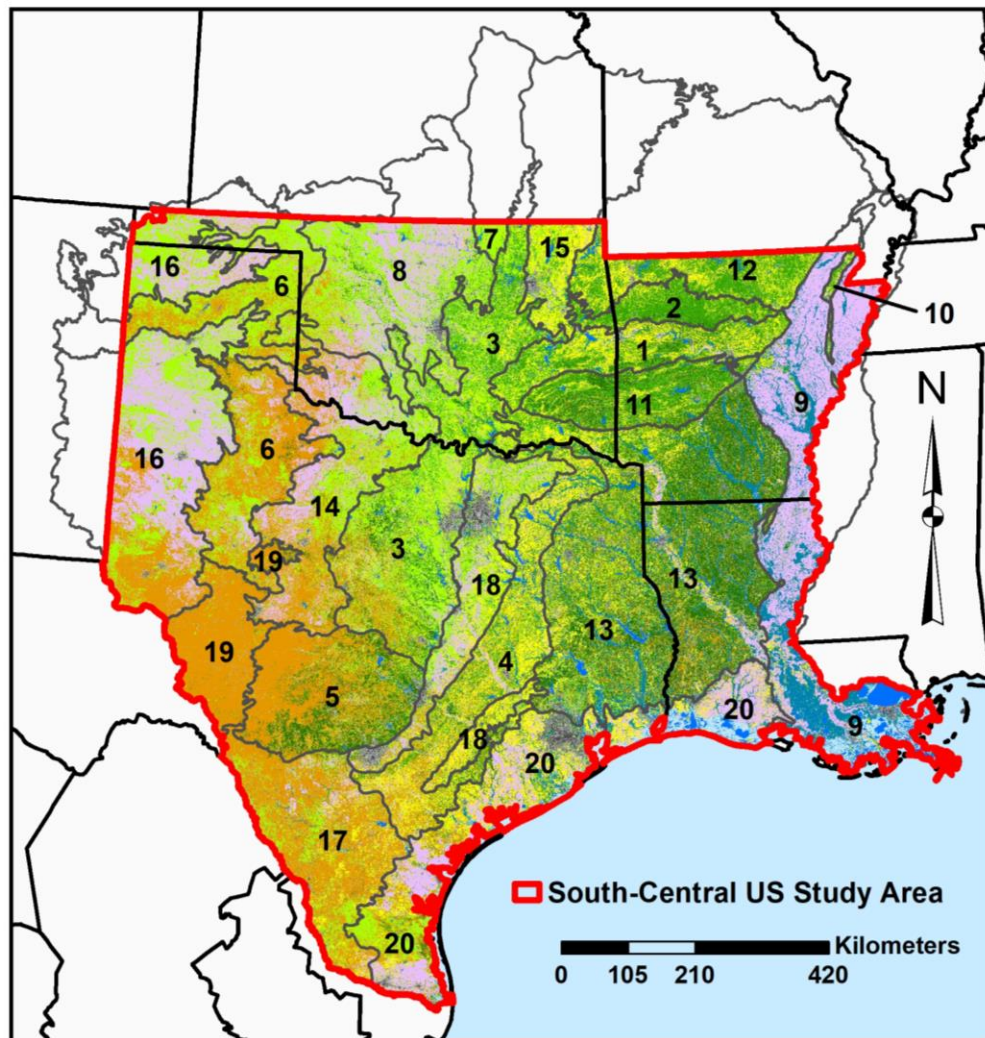
A novel land cover utilization index (LCUI) was developed to identify under-utilized land cover types within the identified MaxEnt core habitat regions for individual species of milkweeds for the South-Central US study area. The South-Central US study area was comprised of Arkansas and the majority of Oklahoma, Texas, and Louisiana. Additional boundaries to the west in Texas and Oklahoma represent the major distributional division between *A. asperula capricornu* to the east and *A. asperula asperula* to the west (Woodson 1954 and Results). The natural boundary of the Mississippi River to the east in Louisiana serves to exclude the eastern portion of the Southeastern USA Plains Level II ecoregion (Fig. III.1). The LCUI was calculated for each 2011 NLCD land cover class (Homer et al. 2015, MLRC 2017) by dividing the percent of milkweed occurrences found within a land cover class (percent land cover used) by the percent of the land cover class available throughout the core habitat region identified in the MaxEnt model (percent land cover available). Thus, $LCUI = (\% \text{ land cover utilized}) / (\% \text{ land cover available})$. Occurrence data were screened to approximately 30 m precision to match the NLCD data by keeping only milkweed locations with at least three decimal places (ca. 90 m precision) for both latitude and longitude decimal degrees and at least four decimal places (ca. 9 m precision) for either latitude or longitude. Individual milkweed occurrences were then spatially filtered to 1 km and weighted to reduce bias from human population density and nearness to roads by land cover class (for details, see Appendix E, Methods). Land cover risk ratings were assigned according to LCUI as: (1) high risk, less than 40% LCUI; (2) moderate risk, $40\% \leq LCUI < 70\%$; and (3) low risk, $\geq 70\%$ LCUI. The LCUI values were color coded for each of the risk ratings and mapped across the core habitat of each milkweed species in the South-Central US region to provide milkweed habitat land cover risk assessments.

Results

Taxonomic data derived from iNaturalist (2018) imagery indicate that *A. a. asperula* strongly dominates to the west and *A. a. capricornu* dominates to the east of a boundary defined by the Pecos River in Texas and longitude 103°N in the Texas and Oklahoma panhandles (Fig. E.1). The eastern boundary of the Mesa de Maya/Black Mesa CEC Level IV ecoregion at the northwestern tip of the Oklahoma panhandle was also used to separate *A. a. asperula* to the west due to their records from this ecoregion nearby in Colorado (Fig. E.2). Using *A. a. capricornu* data east of the boundary (Fig. E.2), the MaxEnt core habitat primarily occupied the southeastern portion of the South-Central Semiarid Prairies from western Oklahoma to central Texas (Fig. III.2). *Asclepias viridis* core habitat occurred along the eastern edge of the South-Central Semiarid Prairies from Kansas to Texas and includes the southern Central Irregular Prairies, the eastern portion of the Ozark Ouachita Appalachian Forests (excluding the mountainous

Modified CEC Level III Ecoregions

Arkansas Valley (1)	Middle Central Great Plains (8)	Southern Central Irregular Plains (15)
Boston Mountains (2)	Mississippi Alluvial Plain (9)	Southern High Plains (16)
Cross Timbers (3)	Mississippi Valley Loess Plains (10)	Southern Texas Plains (17)
East Central Texas Plains (4)	Ouachita Mountains (11)	Texas Blackland Prairies (18)
Eastern Edwards Plateau (5)	Ozark Highlands (12)	Western Edwards Plateau (19)
Eastern Southwestern Tablelands (6)	South Central Plains (13)	Western Gulf Coastal Plain (20)
Flint Hills (7)	Southern Central Great Plains (14)	



2011 National Land Cover Database

Barren Land	Developed, Medium Intensity	Mixed Forest
Cultivated Crops	Developed, Open Space	Open Water
Deciduous Forest	Emergent Herbaceous Wetlands	Pasture/Hay
Developed, High Intensity	Evergreen Forest	Shrub/Scrub
Developed, Low Intensity	Grassland/Herbaceous	Woody Wetlands

Fig. III.1. South-Central US study area for land cover risk assessment analyses with land cover classes of the 2011 National Land Cover Database (Homer et al. 2015, MLRC 2017) and modified CEC (2005) Level III ecoregions. Ecoregions were modified to delete portions non-contiguous with the study area or in Mexico, and add regional divisions for several Level III ecoregions (for details, see Appendix E Methods).

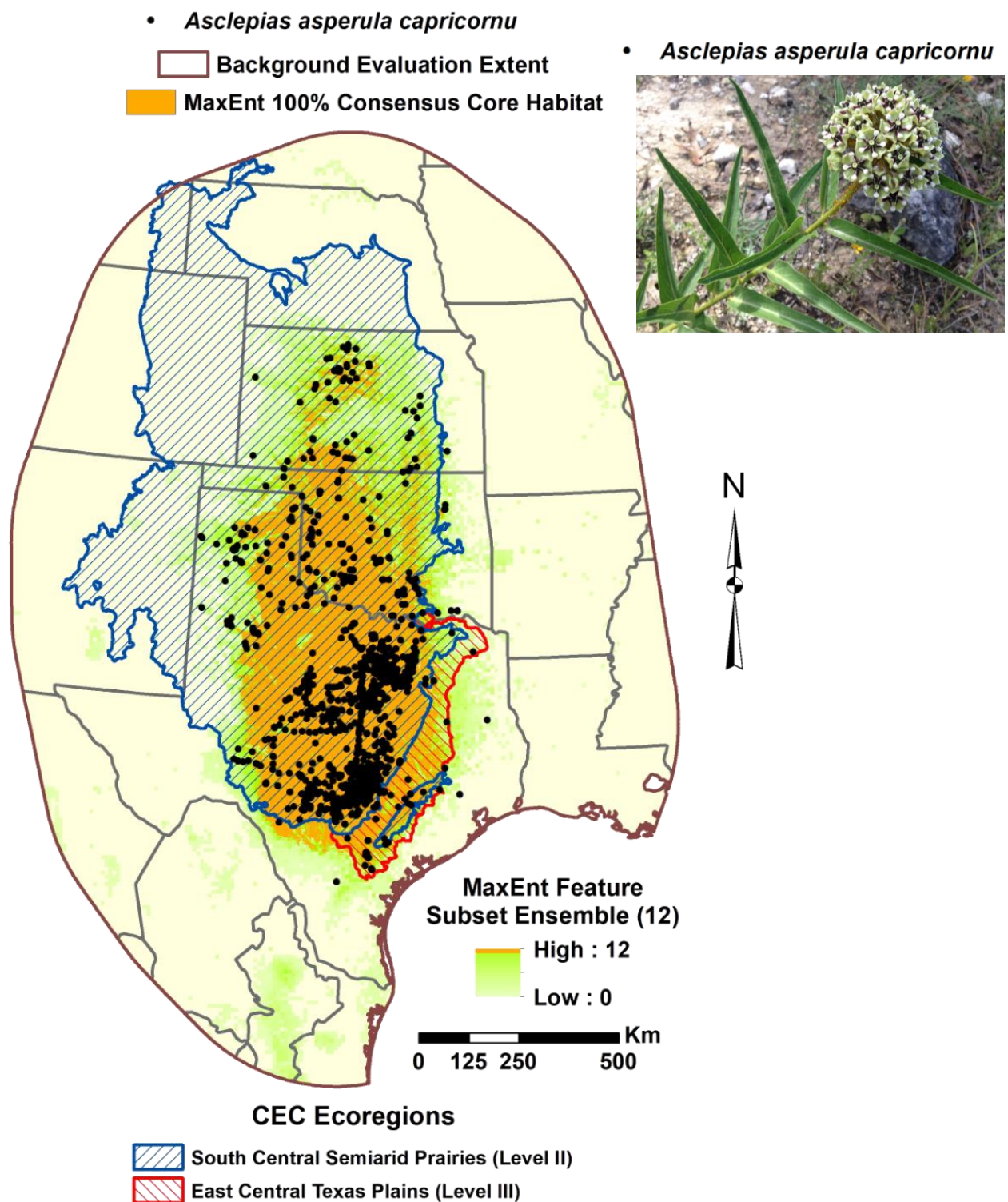


Fig. III.2. *Asclepias asperula capricornu* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of six of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

ecoregions), the western portion of the Southeastern USA Prairies, and the eastern Texas portion of the Texas Louisiana Coastal Plains. Projections for suitable *A. viridis* habitat outside the core range were strongest over the Southeastern USA Plains in Mississippi and Alabama (Fig. III.3). The MaxEnt core distribution of *A. oenotheroides* was concentrated along the southeastern edge of the South-Central Semiarid Prairies and northern portions of the Tamaulipas Texas Semiarid Plain. The *A. oenotheroides* core distribution includes southern portions of the Texas Louisiana Coastal Plain, and portions of various Level II ecoregions in Mexico, including the Dry Gulf of Mexico Coastal Plains and Hills and Interior Depressions (Fig. III.4). The core eastern habitat of *A. latifolia* includes the West-Central portion of the South-Central Semiarid Prairies from southwestern Kansas and southeastern Colorado to eastern New Mexico and northwest Texas, and portions of the northern Chihuahuan Desert. The only milkweed of the four major species with core habitat extending eastwards to Arkansas and western Louisiana is *A. viridis*. Only *A. latifolia* core habitat extended west to include much of the Arizona/New Mexico Plateau and portions of the Colorado Plateaus in southern Utah (Fig. III.5).

The 2011 NLCD land cover classes with low LCUI (high risk) by *A. a. capricornu* include deciduous forest, woody wetlands, cultivated crops and pasture/hay. The *A. a. capricornu* LCUI values for these classes were significantly lower than for other classes (Table III.1, Fig. III.6A). Most of the *A. a. capricornu* high risk land covers occurred over the southeastern portion of the core habitat in the East Central Texas Plains and portions of western Oklahoma (Figs. III.2,6B). For *A. viridis*, high risk land covers with significantly lower LCUIs than other land cover classes were represented by all three forest land types (Deciduous, Evergreen, and Mixed), Woody Wetland, and Cultivated Crops (Table III.1, Fig. III.7A). Main areas of high land cover risk for *A. viridis* included portions of the Texas Louisiana Coastal Plain, and the South-Central Semiarid Prairies of western Oklahoma (Figs. III.3,7B). Cultivated Crops is the only high risk land cover with significantly lower LCUIs for *A. oenotheroides*, mostly occurring along the southern portions of the Texas Louisiana Coastal Plain and the Texas Blackland Prairies. Moderate risk land covers for *A. oenotheroides* with significantly lower LCUIs include Grassland/Herbaceous and Woody Wetlands land cover classes in Cross Timbers and South Texas (Table III.1, Fig. III.8). The two high risk land cover classes with significantly lower LCUI for *A. latifolia* were Deciduous Forest and Cultivated Crops. Shrub/scrub represented moderate risk land cover with significantly lower LCUI (Table III.1, Fig. III.9A). Minimal high risk land cover was found over the core habitat of *A. latifolia* in the South-Central states, but moderate risk land cover was widespread over the southeastern portion of the habitat in the South-Central Semiarid Prairies (Fig. III.5,9B).

Discussion

Taxonomic classification of *A. asperula asperula* and *A. a. capricornu* across the Southwest from iNaturalist (2018) imagery further support the fairly distinctive western and eastern distributions of these subspecies, respectively, as found by Woodson (1954, their Fig. 111) (Fig. E.1). Our data support the suggestion by Singhurst et al. (2015) that species status may be warranted for these taxa based upon clear differences in morphology and distribution (Woodson 1954). Further studies are needed to determine the degree of hybridization in these taxa and the geographic distribution and frequency of the hybrids. Among the four major milkweed species of the South-Central study area, the MaxEnt core distribution of *A. a.*

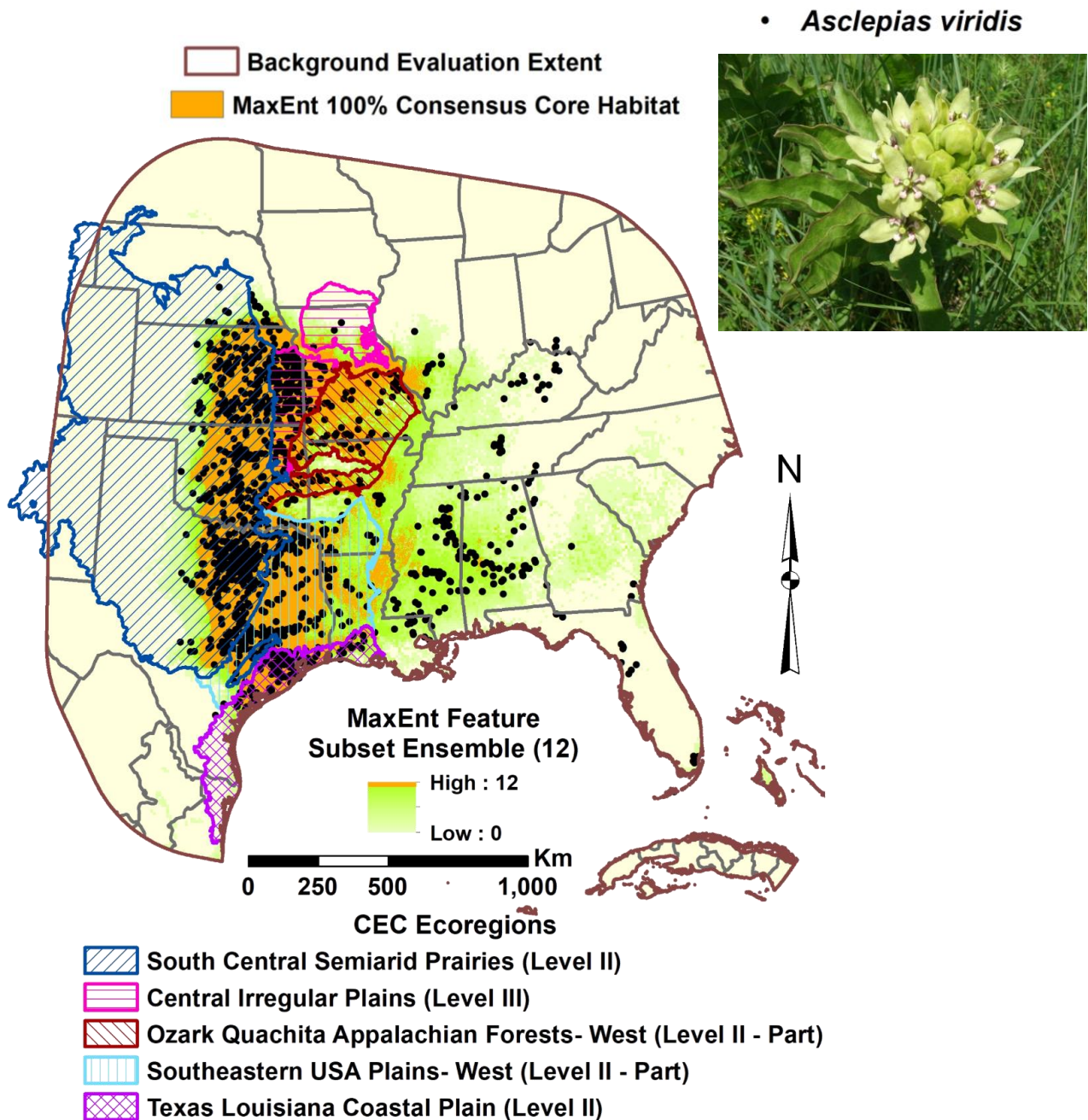


Fig. III.3. *Asclepias viridis* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of eight of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

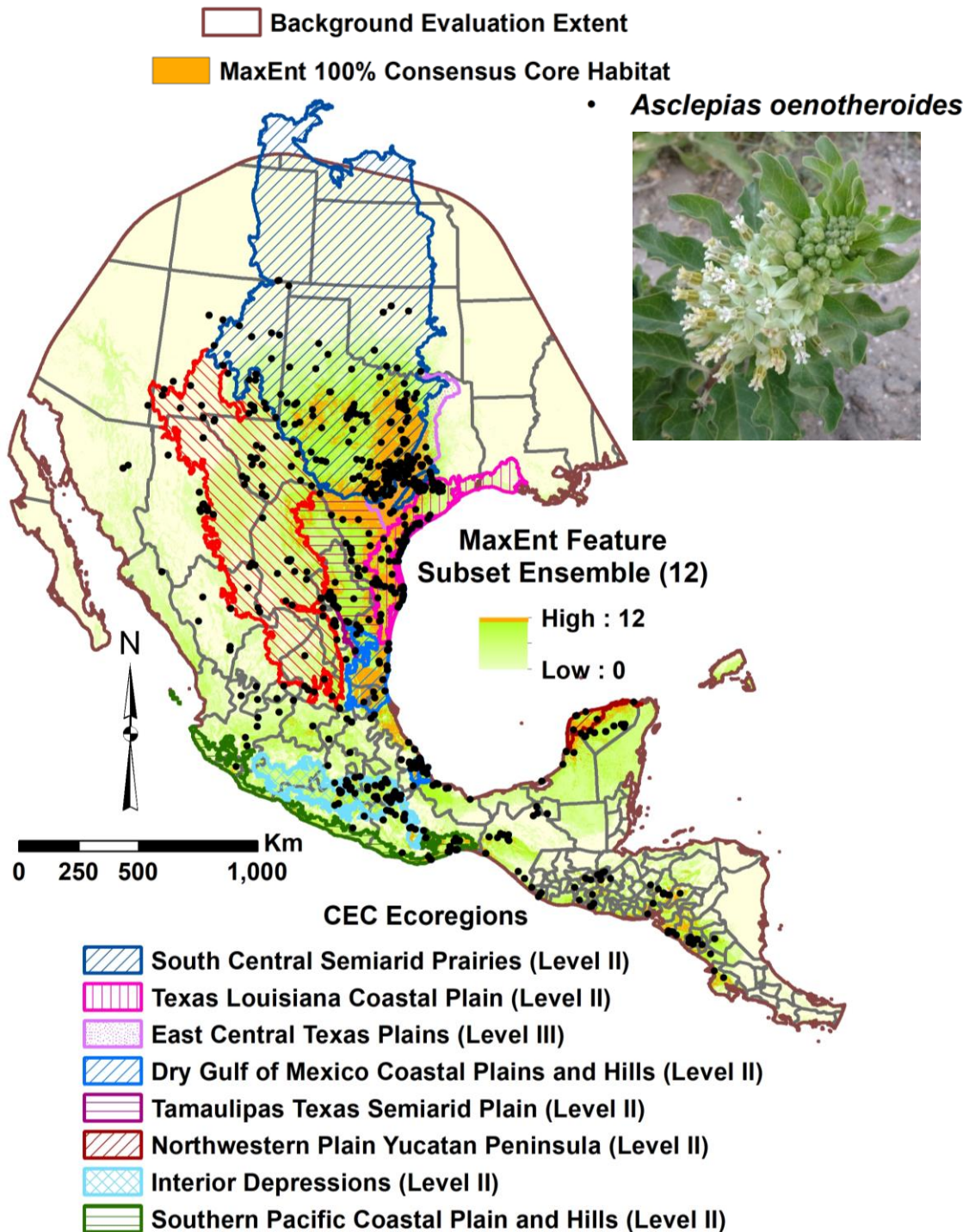


Fig. III.4. *Asclepias oenotheroides* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of eight of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

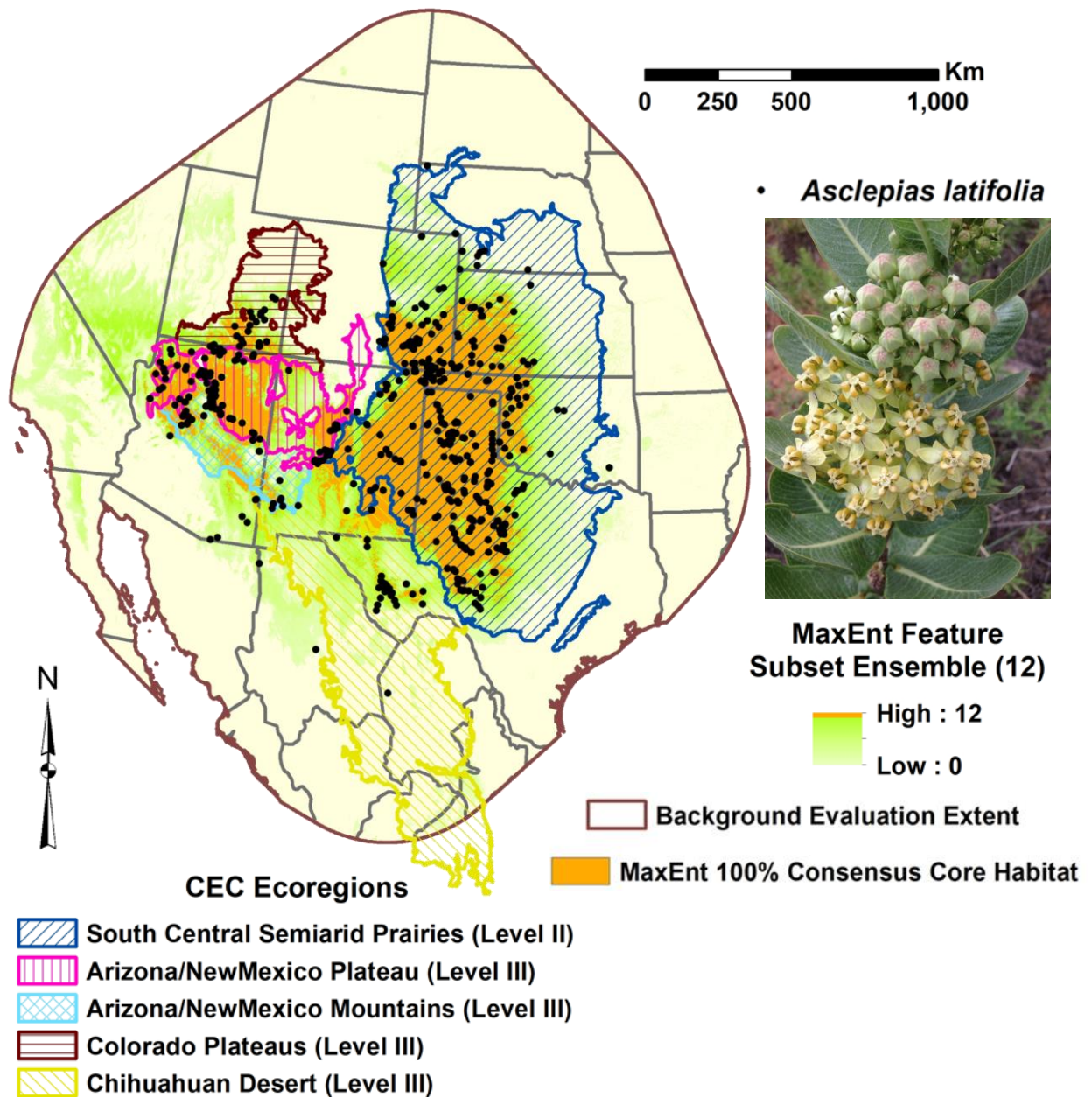


Fig. III.5. *Asclepias latifolia* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of six of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

Table III.1. Mean land cover utilization index (LCUI) over 12 MaxEnt modeled core habitats in South-Central US study area for four milkweed species (*Asclepias* spp).

2011 National Land Cover Database (NLCD) Land Cover Class	Mean Land Cover Utilization Index (%; n = 12; \pm SD) ^a			
	<i>A. asperula</i>		<i>A.</i>	
	<i>capricornu</i>	<i>A. viridis</i>	<i>oenotheroides</i>	<i>A. latifolia</i>
Cultivated Crops	9.9 \pm 0.7i*	21.0 \pm 0.8h*	8.14 \pm 0.7 h*	27.1 \pm 0.9e*
Deciduous Forest	32.9 \pm 2.3f*	35.8 \pm 1.8f*	83.6 \pm 8.7 d*	1.9 \pm 0.9f*
Developed, Low Intensity	1107.6 \pm 56.1a*	533.5 \pm 24.5b*	586.7 \pm 64.8 a*	NA
Developed, Medium Intensity	NA	NA	196.4 \pm 32.0 b*	NA
Developed, Open Space	518.3 \pm 4.2b*	561.8 \pm 18.8a*	521.1 \pm 23.2 a*	1240.9 \pm 50.0a*
Emergent Herb. Wetlands	Na	100.1 \pm 33.0de	NA	NA
Evergreen Forest	120.3 \pm 7.1c*	30.5 \pm 5.1g*	132.8 \pm 5.3 c*	97.1 \pm 20.7b
Grassland Herbaceous	70.8 \pm 2.3e*	111.9 \pm 10.0c*	53.2 \pm 2.8 f*	71.2 \pm 3.3c*
Mixed Forest	NA	26.0 \pm 3.5g*	NA	NA
Pasture/Hay	11.6 \pm 0.8h*	100.2 \pm 6.7d*	73.4 \pm 7.8 e	NA
Shrub/Scrub	97.0 \pm 1.6d*	76.1 \pm 6.6e*	86.0 \pm 6.8 c*	56.4 \pm 4.0d*
Woody Wetlands	24.8 \pm 4.9g*	21.1 \pm 3.4h*	43.4 \pm 2.9 g*	NA

^an = 12 MaxEnt model areas of overlap with 100% consensus core habitat MaxEnt model. Means within a replicate with a different letter are significantly different (P < 0.05; Kruskal-Wallis Rank Sum Test followed by Pairwise Wilcoxon Rank Sum Test). Means with asterisks signify Land Cover Value Index (LCVI = LCUI – 100) for class is significantly different from zero, indicating land cover utilization is different from expected (LCUI = 100) (P < 0.05; Paired Wilcoxon Signed Rank Test).

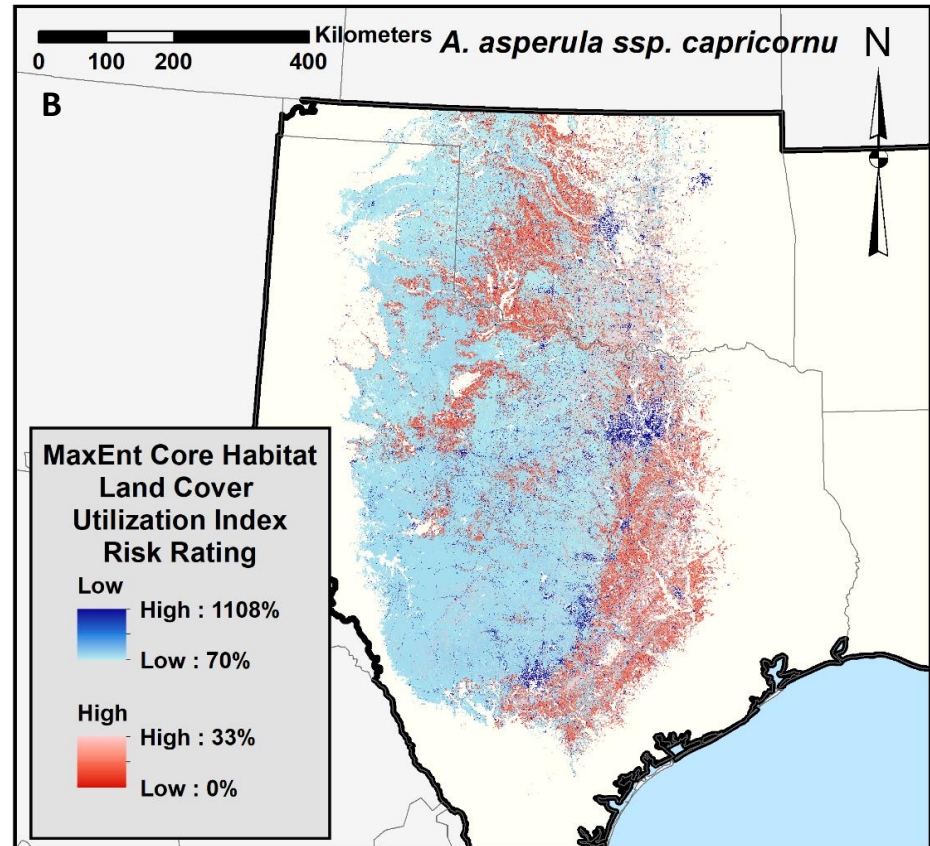
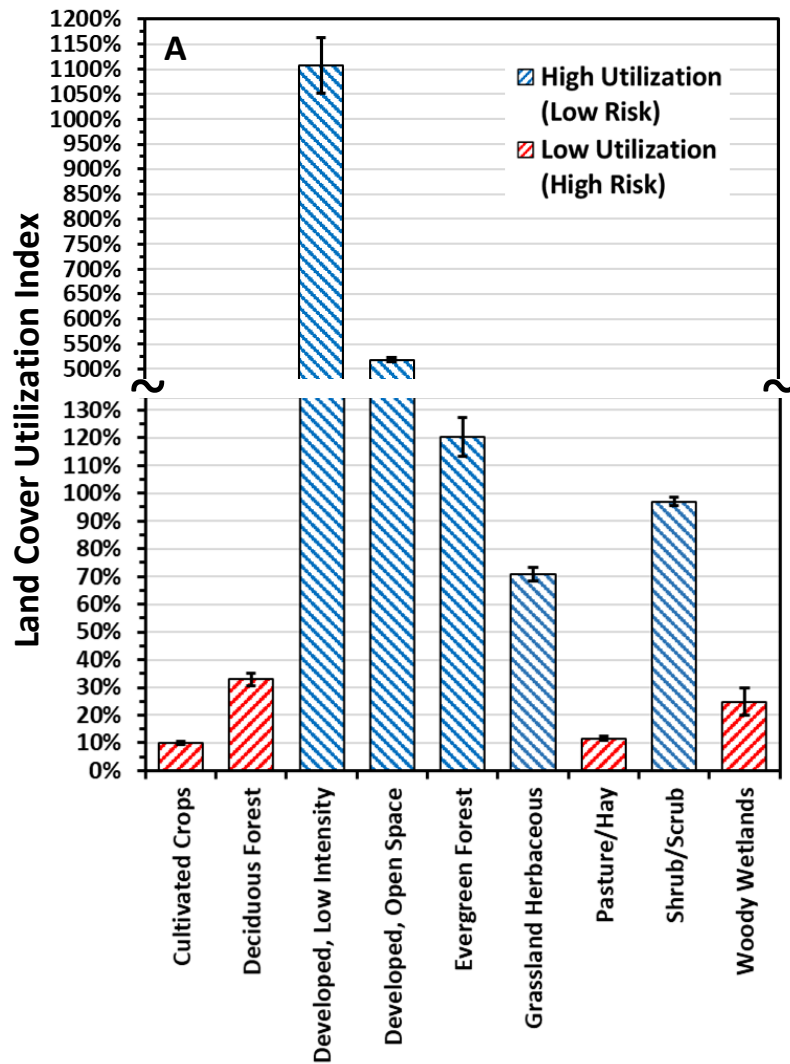


Figure III.6. *Asclepias asperula capricornu* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

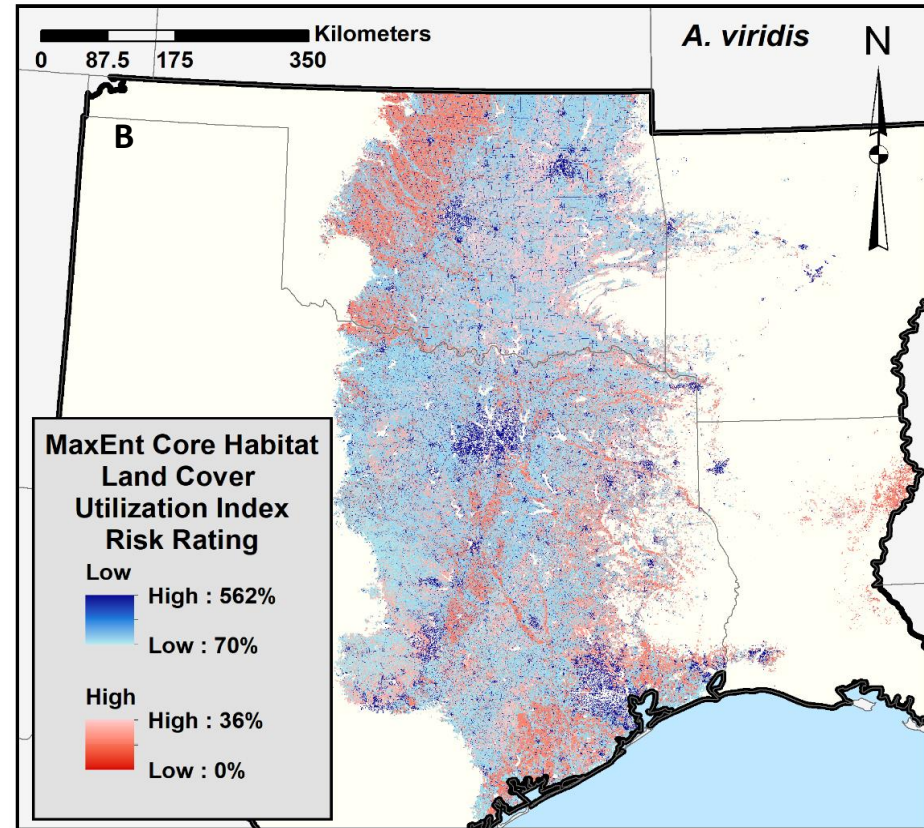
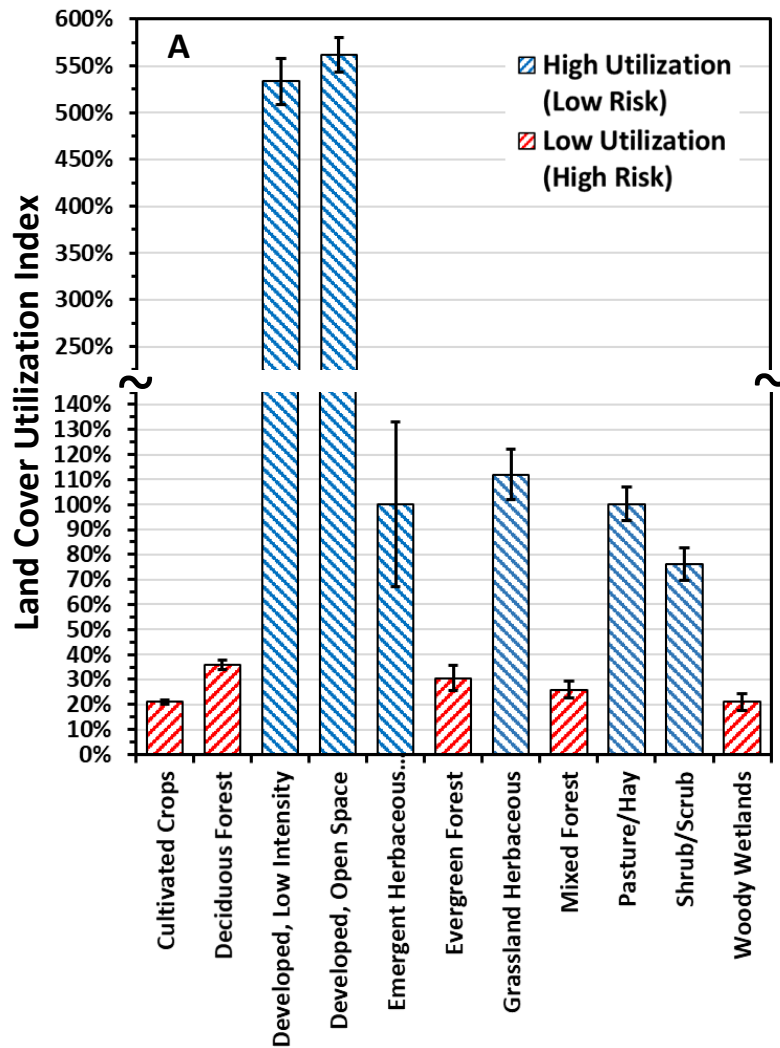


Figure III.7. *Asclepias viridis* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

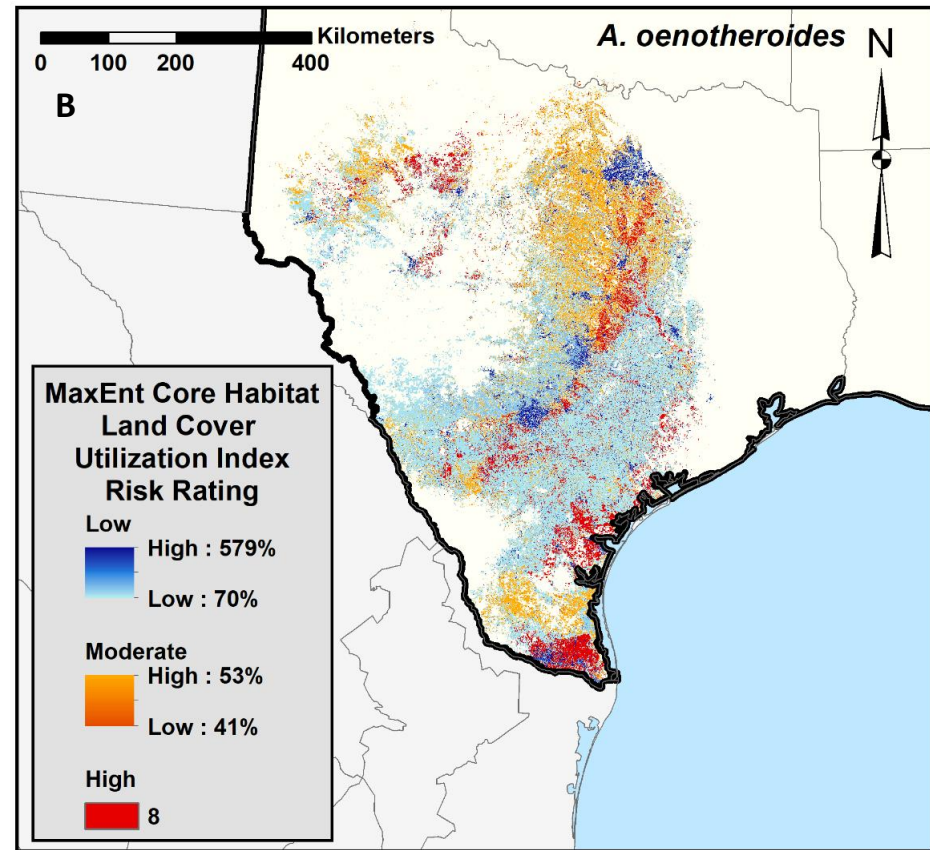
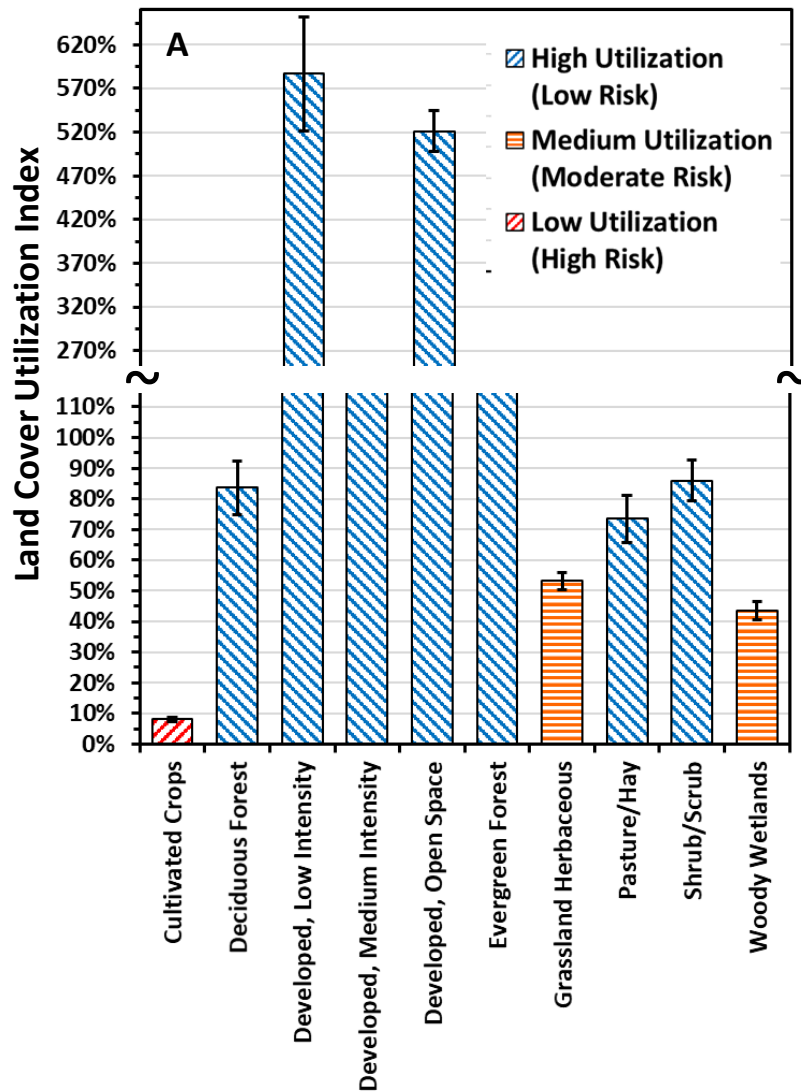


Figure III.8. *Asclepias oenotheroides* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

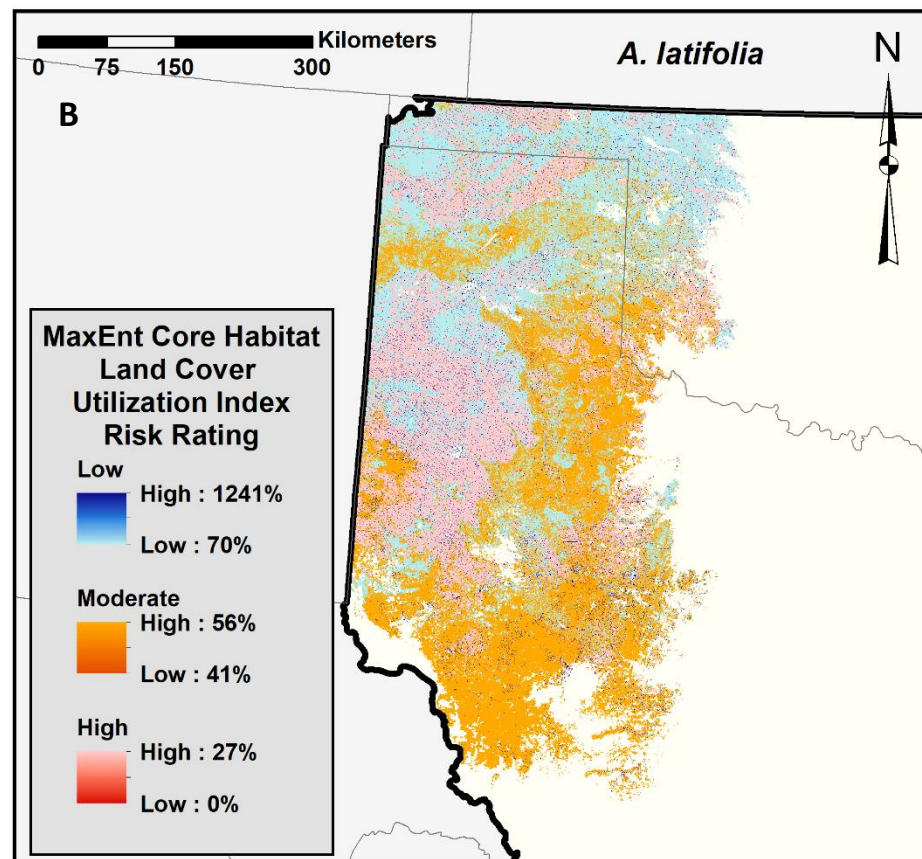
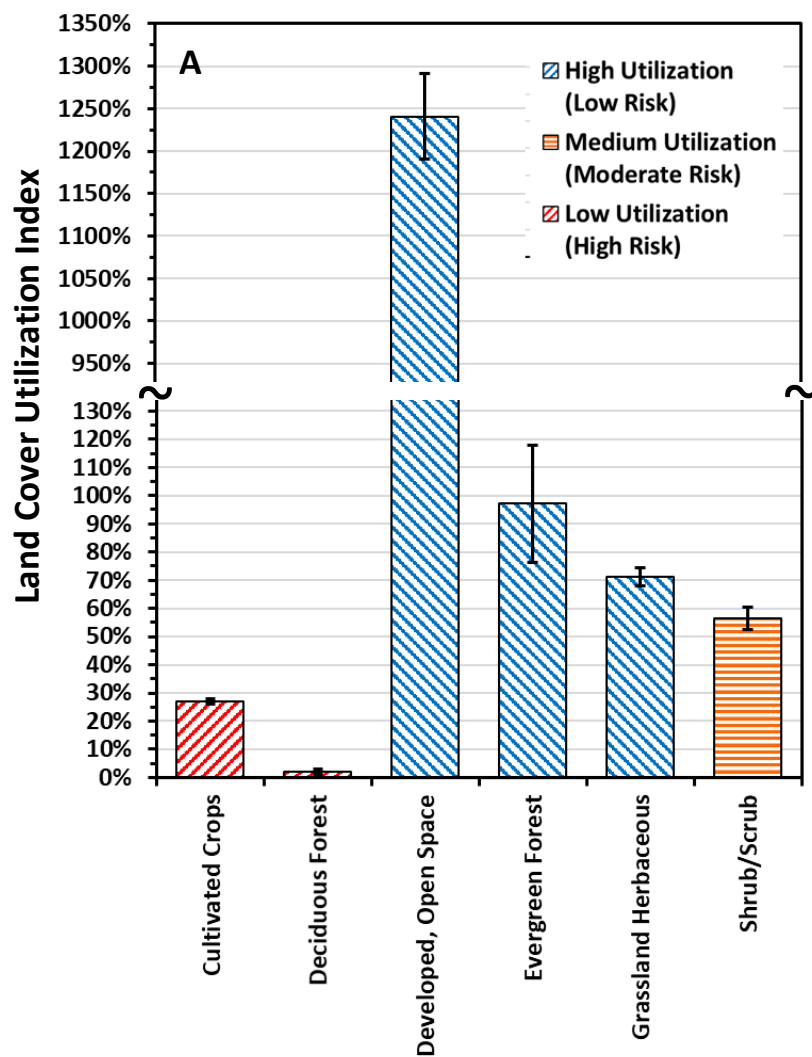


Figure III.9. *Asclepias latifolia* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

capricornu most dominates the South-Central portion of the South-Central Semiarid Prairies from central Texas to western Oklahoma. In contrast, the core distribution of *A. viridis* extends further north to Kansas and to the east in Missouri, Arkansas, and Louisiana, overlapping with that of *A. a. capricornu* along the southeastern edge of the South-Central Semiarid Prairies. In contrast to the 0.5 probability contour for the MaxEnt distribution of *A. viridis* from Lemoine (2015), our modeled MaxEnt core distribution of *A. viridis* excludes much of the Midwest (e.g., most of Illinois and all of Indiana) and extends further south to Texas and Louisiana. These differences may arise from a larger number of *A. viridis* observations to the west and south from our additional data sources, such as iNaturalist (2018) and Texas field survey data. Among the four South-Central milkweeds, *A. viridis* is the most important species for eastern Texas, eastern Oklahoma, and parts of western Arkansas and Louisiana. In the Tamaulipas Semiarid Plain of South Texas and portions of various ecoregions in Mexico, *A. oenotheroides* is the most important of the four South-Central milkweeds. To the west, *A. latifolia* MaxEnt core habitat dominates among the four South-Central milkweeds, uniquely occupying the southwestern edge of the South-Central Semiarid Prairies in western Texas, western Oklahoma, and New Mexico and extending even further west to northern Arizona and southern Utah.

Cultivated Crops represents one of the greatest land cover threats to all four species of South-Central milkweeds due to their poor tolerance of cultivation. The Pasture/Hay land cover class represents much of the land cover risk over the core distribution of *A. a. capricornu*, but does not represent a risk for *A. viridis* or *A. oenotheroides* (it does not occur over *A. latifolia* core habitat). The lower utilization of Pasture/Hay by *A. a. capricornu* may be related to a generally lower abundance of this species over the eastern portion of its range where Pasture/Hay is more abundant rather than to any lower suitability of Pasture/Hay for this milkweed compared to others species, such as *A. viridis* (Figs. III.1-3). The Grassland Herbaceous land cover represented a moderate land cover risk only to *A. oenotheroides*. More open, high disturbance habitats, such as roadsides and waste places, are favored by *A. oenotheroides* (Woodson 1954, Lehman et al. 2005). Lower disturbance leading to less open areas in contemporary Grassland Herbaceous land cover may disfavor *A. oenotheroides*. Shrub/scrub land cover represented a moderate risk for *A. latifolia* core habitat. Hart et al. (2000) noted that *A. latifolia* was more common along trails and roadsides than rangelands in west Texas, which are typically Shrub/Scrub land covers. Similar to *A. oenotheroides*, lack of sufficient contemporary disturbance in natural Shrub/Scrub land covers may contribute to lower utilization by *A. latifolia*.

Highly utilized land covers with low risk for all four milkweeds included various anthropogenic types, such as Developed-Low Intensity and Developed-Open Space, which are associated with roadsides and urban areas. Regular mowing disturbance in these anthropogenic areas probably favors growth of milkweeds similar to how regular mowing of grasslands in Oklahoma can increase the cover of broadleaf species such as *A. viridis* and suppress grasses such as invasive KR bluestem (*Bothriochloa ischaemum*) (Dee et al. 2016). In addition, roadside mowing at the right time during the early summer can stimulate additional late summer/fall *A. viridis* resprout growth with adequate rainfall, potentially benefiting fifth generation monarch larvae (Baum and Mueller 2015). However, mowing of milkweeds in urban and roadside areas during the spring and late summer/fall monarch breeding seasons can make them much more risky than un-mown areas for successful monarch larval development. Consequently, much of the highly utilized roadside and urban milkweed habitats could potentially be an ecological trap

for monarch larvae if mowed during the monarch breeding seasons. Monarch Joint Venture (2016) has suggested periods to avoid mowing to benefit monarchs for different regions, including the South (below 35°N), where mowing is to be especially avoided during March through June, benefiting the spring migrants and first generation larvae, and early August to October, benefiting the fall migrants and fifth generation larvae.

Ecoregions with areas of high Cultivated Crop cover overlapping core milkweed habitats should be a major focus for mitigating landscape risk for milkweeds. Level III ecoregions with high Cultivated Crop cover for *A. a. capricornu* core habitat in Texas and Oklahoma include the Texas Blackland Prairies, Southern Central Great Plains, and Middle Central Great Plains. High Cultivated Crop areas for *A. viridis* core habitat include the Middle and Southern Central Great Plains in Oklahoma, Texas Blackland Prairies, and the Western Gulf Coastal Plain in eastern Texas. High Cultivated Crop cover over *A. oenotheroides* core habitats include the Texas Blackland Prairie, Southern Central Great Plains, portions of the Southern Texas Plains, and much of the southern Western Gulf Coastal Plain. The highest risk from Cultivated Crop cover for *A. latifolia* occurs in the Southern High Plains in the western Texas panhandle. There is also widespread moderate risk to *A. latifolia* from Shrub/Scrub land cover that is probably related to inadequate contemporary disturbance regimes in the Western Edwards Plateau and Eastern Southwestern Tablelands in West Texas.

An example of a potential approach for mitigation of landscape risk to milkweeds and monarch butterflies for the South-Central US study area is the Southern Great Plains (South Central) Habitat Strategy for enhancing monarch butterfly habitat in Kansas, Oklahoma, and Texas, which was developed by the USDA NRCS (2016a) in cooperation with the USFWS. This strategy incorporates the USDA NRCS Monarch Butterfly Habitat Conservation Plan (MBHP; USDA NRCS 2015) and prioritizes incentivization for the conservation and protection of milkweed stands in the southern Great Plains. The main strategy involves promotion of converting invasive monoculture grasslands to species rich native grasslands and the implementation of grazing systems that maximize species richness, including nectar species. Various management practices were identified with the potential to increase milkweed and nectar plant populations in grasslands, including brush management, prescribed burning, prescribed grazing, and mowing. Within Texas, the MBHP focuses on counties mostly within the Texas Blackland Prairies and provides financial assistance for eligible private landowners to promote growth of milkweeds and nectar plants in the spring and fall (USDA NRCS 2016b) using prescribed conservation practices for the southern plains and prairies (USDA NRCS 2018). Related to these monarch conservation efforts with funding from the National Fish and Wildlife Foundation, the Texas Soil and Water Conservation Board (2016) Monarch Habitat Project promotes growth of pollinator friendly plants within their Monarch Restoration Corridor covering most of the eastern half of Texas. Additional funding and focus of milkweed habitat restoration activities within the identified high land cover risk areas in Texas and Oklahoma could increase milkweed resources available for monarch butterfly larvae in the critical spring first generation and fall fifth generation.

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SECTION IV: MONARCH LARVAL SEASONALITY AND HOST PLANT USE BY PHENOREGIONS

Introduction

The South-Central US region is critical for development of monarch spring first generation and the late summer and fall fifth generation larvae (Oberhauser et al. 2017). An understanding of the regional seasonal activity and host use of monarch larvae within the South-Central US states of Oklahoma, Texas, Louisiana and Arkansas can guide the prioritization of the timing and location of monarch conservation activities. However, there are few published reports of milkweed host use in Texas.

For the spring generation in Texas, the three native milkweed species most commonly reported from Journey North (2017a) and the Monarch Larva Monitoring Project (MLMP 2017) include *A. a. capricornu*, *A. viridis*, and *A. oenotheroides*. Calvert (1996) reported monarch eggs and larvae on *A. oenotheroides* from 29 March to 7 April, 1995 in south central Texas in a pasture near Luling. All three of these species are herbaceous perennials blooming mostly from spring to summer (Woodson 1954). Lynch and Martin (1993) monitored monarch larvae through the fifth instar on *A. amplexicaulis* (clasping or blunt-leaf milkweed) and *A. viridiflora* (green comet milkweed) from 19 April to 7 May 1985 one mile east of Ore City in northeast Texas. Over a similar time period, they also monitored larval populations on *A. viridis* at two locations in Texas and two in Louisiana and *A. a. capricornu* at two locations in Texas, and also found that fifth instars were successfully produced by early May at most locations. Additional milkweeds used in the spring in natural areas of Texas include *Asclepias incarnata*, *A. texana* (Texas milkweed) (Berman and Baum unpublished), and *A. tuberosa* (iNaturalist 2017). Milkweeds reported as used by monarch larvae in Texas home garden settings during the spring or summer include *A. angustifolia* (Arizona milkweed), *A. curassavica*, *A. fascicularis* (California narrowleaf milkweed), *A. incarnata*, *A. syriaca*, *A. texana*, and *A. tuberosa* (Journey North 2017a, iNaturalist 2017).

During the fall in Texas, Calvert (1999) surveyed roadsides along an 800 mile east/west transect extending from Jasper to Ozona from 27 September to 13 November, 1996. He reported that above average rainfall produced flowering in four species of milkweed *Asclepias viridis*, *A. a. capricornu*, *A. oenotheroides*, and *A. latifolia* (in order of abundance). He found the greatest abundance of monarch eggs and larvae in the fall on *A. latifolia*, followed by *A. a. capricornu*, *A. viridis*, and *A. oenotheroides*. The number of eggs and monarch larvae on *A. latifolia* was three times higher than on *A. viridis* and two times higher than on *A. a. capricornu*. Milkweed species were distributed continuously throughout the transect with some exceptions. He found that almost always one milkweed species occupied each roadside patch. In rare occasions, two species were found within one patch. For example, *A. latifolia* and *A. a. capricornu* were present in the same area in West Texas, and *A. oenotheroides* occurred with *A. viridis* in East-Central Texas. Calvert (1999) suggested that because of this spatial separation of milkweed species, the oviposition data may not show monarch preferences among the milkweed species, but more likely reflect the presence of certain species within a certain geographic area. Batalden and Oberhauser (2015) found a preference for monarch oviposition on *A. curassavica* over *A. a. capricornu* in the fall (but not spring) where both species were present together in the vicinity of

San Antonio, Texas. Additional milkweeds reported as used by monarchs in Texas during the fall include *A. incarnata* and *A. perennis* (aquatic milkweed) (Berman and Baum, unpublished data). Winter breeding of monarchs in South-Central and coastal Texas mostly occurs on home garden plantings of introduced *A. curassavica* (Batalden and Oberhauser 2015).

The objectives of this study were to (1) summarize monarch larval pupal occurrence data by week and location within four phenoregions defined by USDA Plant Hardiness Zones (PHZs; USDA Agricultural Research Service [ARS] 2012) and (2) summarize milkweed host usage across different phenoregions.

Methods

Monarch larval occurrence and host data throughout the South-Central US study area (see Section III, Methods) were obtained from a variety of sources, including iNaturalist (2017), Journey North (2017a, 2017b), Monarch Larva Monitoring Project (MLMP; 2017), and Texas survey data from our laboratory and various collaborators (Janice Bush, University of Texas at San Antonio, 2017; David Berman, Oklahoma State University, 2017). These data were grouped into USDA PHZs for mapping and summary across weeks of the year. Use of hosts in more heavily populated areas were down-weighted to yield a monarch immature population index for each host in each phenoregion.

Results

Monarch larvae were reported throughout most of the South-Central US study area in both spring and summer/fall (Fig. IV.1). Four monarch larval phenoseasons of winter, spring, summer, and fall were identified for the most southern PHZ 9a of southern Texas (Fig. IV.2). The winter larval phenoseason became less detectable in progressively northern PHZs. Most larval activity was in the spring phenoseason across all phenoregions. Spring larval activity peaked progressively later in more northern PHZs. The summer phenoseasons was relatively weak across all PHZs. The fall phenoseason had the second most larval activity compared to the fall phenoseasons.

The most heavily utilized milkweed hosts by PHZs were as follows: (1) *Asclepias latifolia* in the northern PHZs 7a/7b (Tulsa, Oklahoma City); (2) *A. tuberosa*, *A. asperula capricornu*, *A. viridis*, *A. latifolia*, and *A. curassavica* in PHZ 8a (Dallas/Forth Worth); (3) *A. oenotheroides* and *A. viridis* in PHZ 8b (Austin/San Antonio); (4) *A. tuberosa*, *A. viridis*, and *A. curassavica* in PHZ 9a (Houston); and *A. oenotheroides*, *A. curassavica*, and *A. tuberosa* in PHZs 9b/10a (Corpus Christi/Brownsville).

Discussion

Monarch larval activity in the South-Central US is strong over all of the phenoregions for both the spring and fall phenoseasons. There were clear differences in monarch larval host plants across the phenoregions. Further analyses are needed to distinguish monarch host utilization among phenoseasons and between urban and rural areas. These results indicate that monarch conservation measures in the South-Central region, such as the USDA NRCS (2015) Monarch Butterfly Habitat Development Plan, should focus on both the spring and fall phenoseasons and

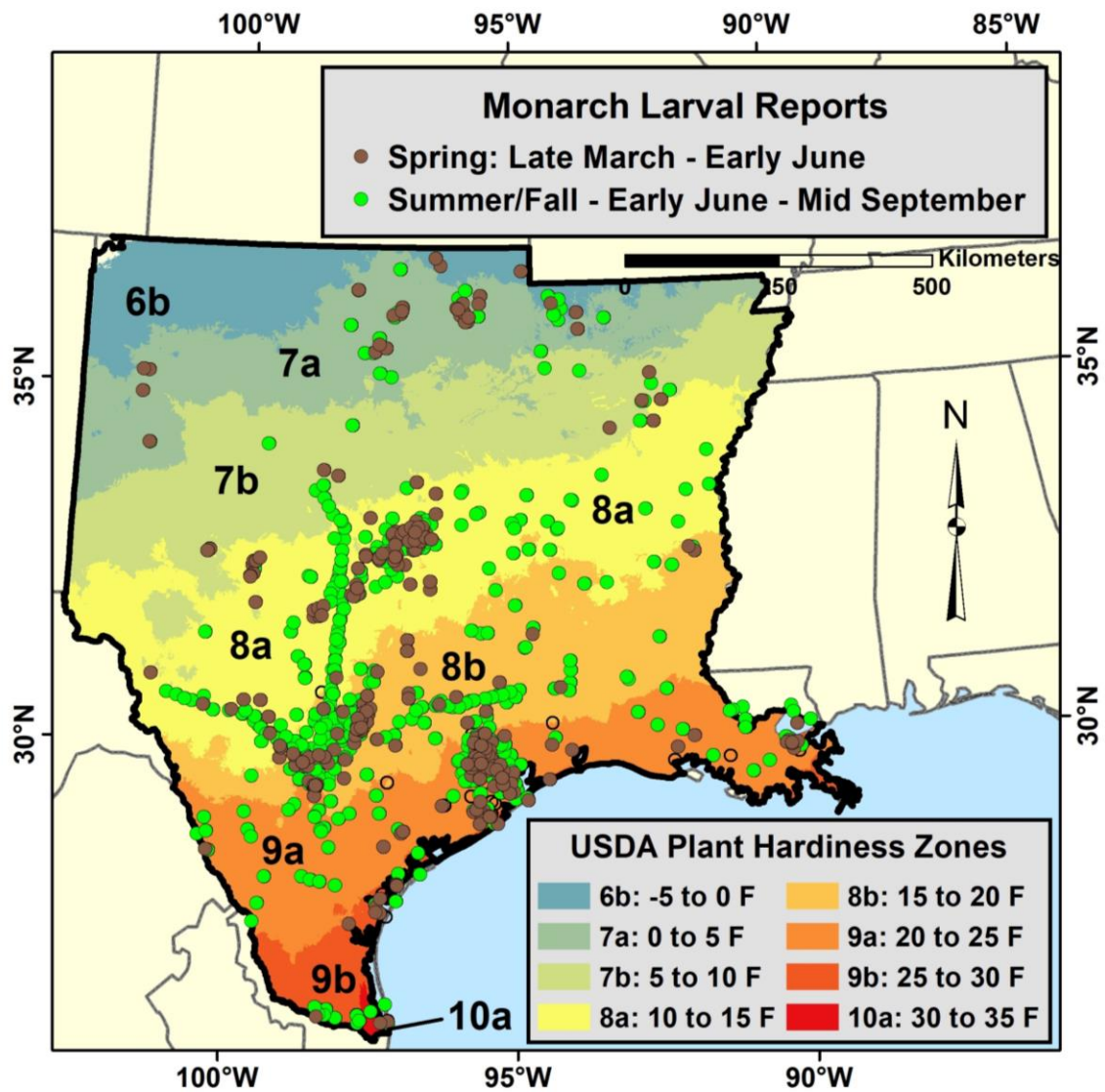


Fig. IV.1. Monarch larval reports for spring first generation and later summer/fall generations in South-Central US study area within USDA Plant Hardiness Zones.

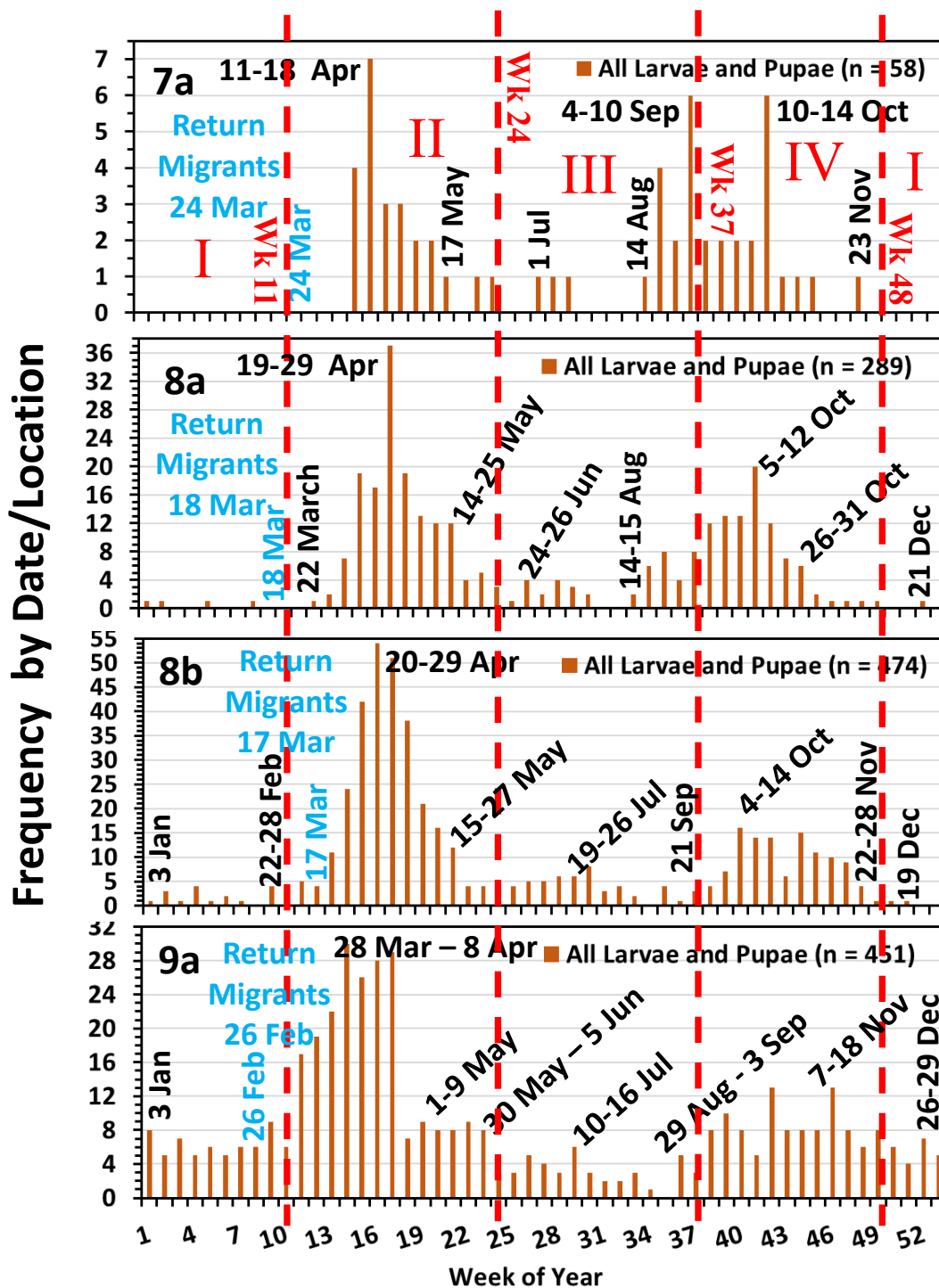


Fig. IV.2. Monarch larval reports for week of year across four main USDA Plant Hardiness Zones in the South-Central US study area showing phenoseasonal divisions of winter (I), spring (II; first generation larvae); summer (III), and Fall (IV; fifth generation larvae).

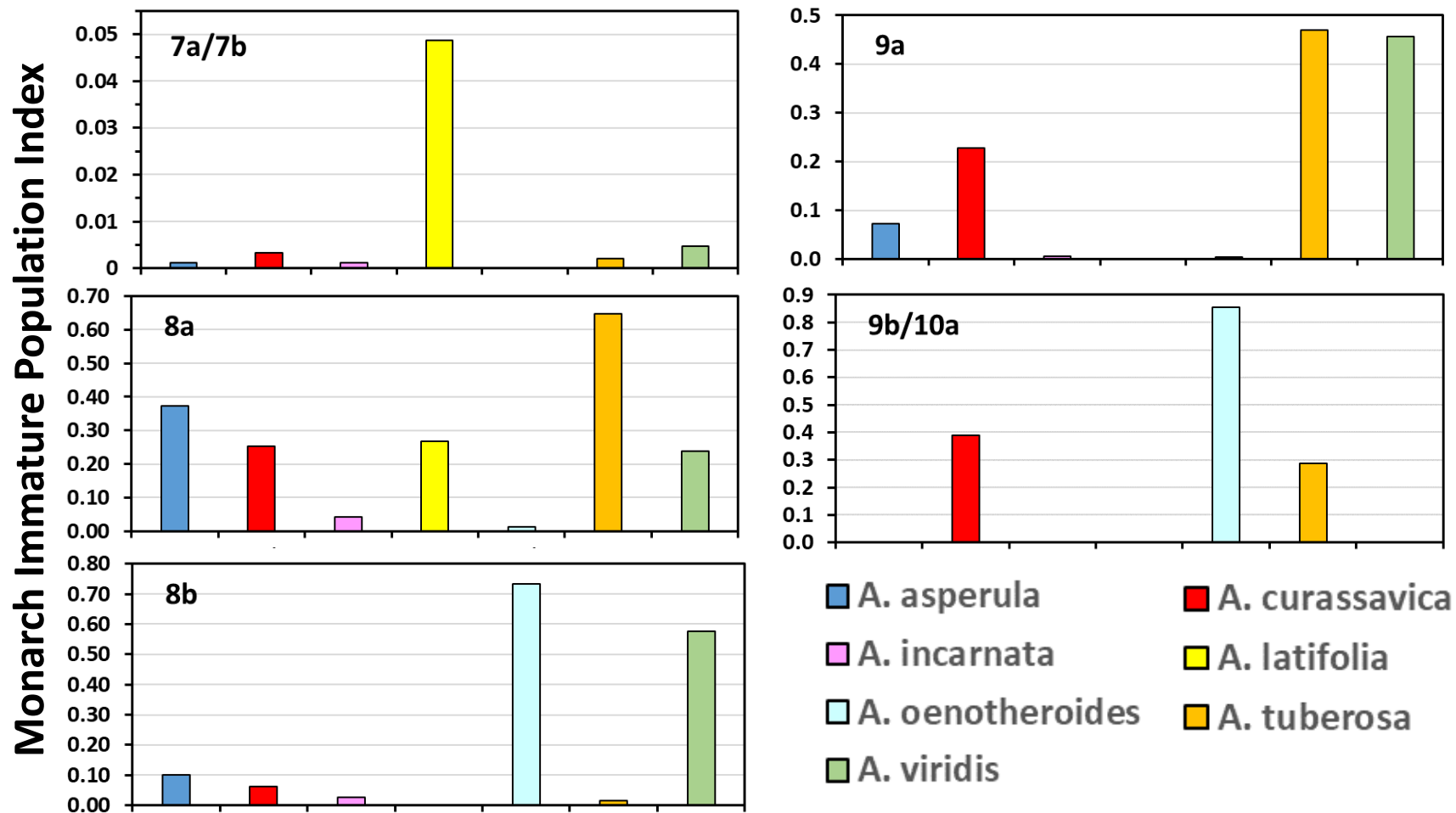


Fig. IV.3. Monarch larval reports on various *Asclepias* host plants year across USDA Plant Hardiness Zones in the South-Central US study area (Fig. IV.1).

target different native milkweeds across the phenoregions. For instance, *A. latifolia* is more important in the northern phenoregions, mostly in the western portion of the study area (see Section III, Results, Fig. III.5). *Asclepias oenotheroides* is one of the more heavily utilized milkweed hosts in the central (PHZ 8b) and southern (PHZ 9a, 9b/10a) phenoregions. *Asclepias viridis* has the most consistent monarch host use across the most (three) phenoregions, while *A. asperula capricornu* host use is mostly limited to the central (PHZ 8a) phenoregion. The milkweeds *A. curassavica*, *A. tuberosa*, and *A. incarnata* are common ornamentals in the study area and most of their larval records are probably from urban areas.

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SECTION V: MONARCH SPRING AND FALL NECTAR PLANT HABITAT MODELS AND LAND COVER RISK ASSESSMENT

Introduction

A major anthropogenic factor in the large decline in the eastern population of monarch butterflies has been loss of Midwest milkweed resources for summer breeding due to changes in agricultural land use (Oberhauser et al. 2017, Pleasants et al. 2017, Thogmartin et al. 2017, Stenoien et al. 2018). Anthropogenic mortality during the fall migration may also be important in the decline (Badgett and Davis 2015, Ries et al. 2015, Inamine et al. 2016, Agrawal and Inamine 2018), such as from roadkill (McKenna et al. 2001, Kantola et al. 2019) and mosquito spraying (Tracy et al. 2019). The population decline may also be increased by limitation of nectar resources through anthropogenic land use change especially during the southern portion of the fall migration through Texas and northern Mexico (Brower et al. 2006). A decrease in fall migration nectar availability might be expected to produce decreases in lipid reserves for monarch arriving at the overwintering sites, but lipid reserves of arriving monarchs have been high and there has been no recent decline (Stenoien et al. 2018). However, lower lipid reserves in migrating monarchs may weaken them enroute and make them more susceptible to other mortality factors, such as roadkill, predation, or cold stress, eliminating most of the monarchs with lower lipid reserves before their successful arrival to the Mexican overwintering sites.

There is a lack of landscape risk assessments for nectar species which could guide efforts on where to provide additional nectar resources for monarchs, especially where they may be most limited over the southern migration route in the South-Central US. We utilize 10 common native nectar plants favored by monarchs as indicator species (c.f., Lawler et al. 2003) for a monarch nectar resource landscape risk assessment over the South-Central US. Seven of the nectar plants are primarily important for the fall migrating adults, and three species are important for the spring and sometimes fall migrating adults.

The objectives of this study were to (1) develop range-wide niche models for ten indicator nectar plants species of the South-Central US, and (2) develop a land cover risk assessment over the modeled core niches of each nectar plant species for the South-Central US region. For the last objective, we used a land cover utilization index to score the percent occupancy of 2011 NLCD land cover types by each nectar plant species.

Methods

Occurrence data throughout the entire North America ranges for ten native indicator species nectar plants with high to very high value for monarch butterflies in the South-Central US (Table V.1) were obtained from a variety of sources, including iNaturalist (2018), SEINet (2017), Global Biodiversity Information Facility (GBIF; 2017), and vPlants (2017).

An initial set of 95 environmental variables at 1 km resolution were used in developing MaxEnt niche models, including 57 climatic indices, 14 topographic indices, and 24 soil indices (Table E1). MaxEnt version 3.3.3 models (Phillips et al. 2006) were developed from milkweed locations using the R-software (R Core Team 2017) dismo package (Hijmans et al. 2011). The occurrence locations were thinned by a 10 km spatial filter for reducing sample bias and spatial

Table V.1. Ten native nectar plant species valuable to monarch butterflies in the South-Central US, including flowering periods and distribution.

Nectar Plant Species	Value as Monarch Nectar Plant	General Flower Period ^a	Distribution in Flyway for South-Central US ^b		Source for Nectar Value
			Central Flyway	Coastal Flyway	
<i>Baccharis neglecta</i> (Roosevelt weed)	Very High	Sep-Oct	East and Southwest	West	USDA NRCS 2015a
<i>Baccharis halimifolia</i> (groundsel tree)	Very High ^c	Sep-Oct	--	East	USDA NRCS 2015a, Brown and Cooperider 2011
<i>Baccharis salicina</i> (willow Baccharis)	Very High ^c	Sep-Oct	West	--	USDA NRCS 2015a
<i>Solidago altissima</i> (tall goldenrod)	High	Sep-Nov	East	East	Ajilvsgi 2013
<i>Verbesina virginica</i> (white crownbeard)	High	Aug-Nov	East	East	USDA NRCS 2015a
<i>Conoclinium coelestinum</i> (blue mistflower)	Very High	Jul-Nov	East	East	USDA NRCS 2015a
<i>Viguiera stenoloba</i> (skeletonleaf goldeneye)	High	Jun-Oct	West	--	USDA National Park Service (NPS) 2015
<i>Verbesina enceliodes</i> (golden crownbeard)	Very High	Apr-Oct	West	West	USDA NRCS 2015a
<i>Glandularia bipinnatifida</i> (prairie verbena)	High	Mar-Oct	All	Central	USDA NRCS 2015a
<i>Verbena halei</i> (Texas vervain)	High	Mar-Jun	All	All	USDA NRCS 2015a

^aFlowering periods from USDA NRCS (2015a), Ajilvsgi (2013), Lady Bird Johnson Wildflower Center (2016), Tom Robbins (personal communication).

^bFlyways as defined by Tracy et al. (2019) and general distributions from Kartez (2015).

^cNectar value assumed same as congener.

autocorrelation among the data (Boria et al. 2014). About 10,000 pseudoabsence points were generated for model evaluation within the background evaluation extent, consisting of a 500 km buffer around a convex hull polygon of the occurrence points. The pseudoabsences were buffered at 20 km from the presence points (e.g., Barbet-Massin et al. 2012). The R PresenceAbsence package (Freeman and Moisen 2008) was used with presence and pseudoabsence points for calculating a pseudoabsence (psa) version of the true skill statistic (TSS_{psa}) and area under the curve statistic (AUC_{psa}) using random cross validation. This is in contrast to the standard practice

of utilizing the MaxEnt software default calculation of a background presence (bgp) version of AUC (AUC_{bgp}), which incorporates as absences both background and presence points (not buffered pseudoabsence points). To reduce MaxEnt model complexity and overfitting for improved model generalization (Jiménez-Valverde et al. 2008; Warren and Seifert 2011), we adjusted the MaxEnt beta regularization to two and used only quadratic and hinge features (Tracy et al. 2018). We utilized the random subset feature selection algorithm (RSFSA) to evaluate performance of thousands of MaxEnt models developed from random combinations of subsets of 3 to 25 of the 95 variables, limiting correlation of variables to less than $|0.7|$ (Tracy et al. 2018). The top 12 feature-selected MaxEnt models of six to eight variables each were calibrated to binary presence/absence format using a threshold of maximum TSS_{psa} (Liu et al. 2013). These models were combined using frequency consensus to form an ensemble of models derived from different selected feature subsets (feature subset ensemble). The 100% consensus of the 12 models was defined as the core habitat for the species. Core habitats of each species were identified with portions of Level II and III ecoregions of the Commission for Environmental Cooperation (CEC 2005).

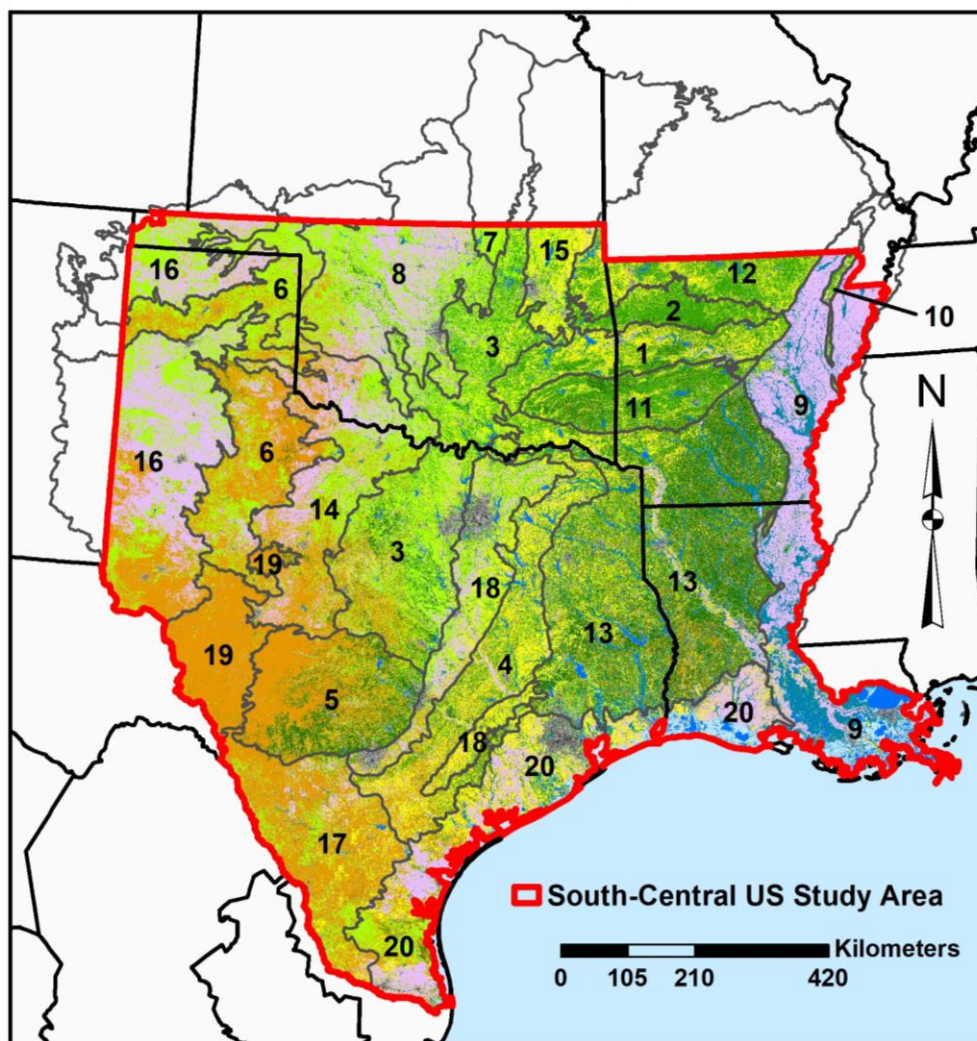
A land cover utilization index (LCUI) was developed to identify under-utilized land cover types within the identified MaxEnt core habitat regions for individual species of nectar plants for the South-Central US study area. The South-Central US study area was comprised of Arkansas and the majority of Oklahoma, Texas, and Louisiana (Fig. V.1; for additional details see Section III, Methods). The LCUI was calculated for each 2011 NLCD land cover class (Homer et al. 2015, MLRC 2017) by dividing the percent of nectar plant occurrences found within a land cover class (percent land cover used) by the percent of the land cover class available throughout the core habitat region identified in the MaxEnt model (percent land cover available). Thus, $LCUI = (\% \text{ land cover utilized})/(\% \text{ land cover available})$. Occurrence data were screened to approximately 30 m precision to match the NLCD data by keeping only nectar plant locations with at least three decimal places (ca. 90 m precision) for both latitude and longitude decimal degrees and at least four decimal places (ca. 9 m precision) for either latitude or longitude. Individual nectar plant occurrences were then spatially filtered to 1 km and weighted to reduce bias from human population density and nearness to roads by land cover class (for details, see Appendix E, Methods). Land cover risk ratings were assigned according to LCUI as: (1) high risk, less than 40% LCUI; (2) moderate risk, $40\% \leq LCUI < 70\%$; and (3) low risk, $\geq 70\%$ LCUI. The LCUI values were color coded for each of the risk ratings and mapped across the core habitat of each nectar plant species in the South-Central US region to provide nectar plant habitat land cover risk assessments.

Results

The MaxEnt core habitat for *B. neglecta* occupied most of Texas, with the exception of far eastern and far western portions of the state, and northeastern Mexico (Fig. V.2A). the southeastern portion of the South-Central Semiarid Prairies from western Oklahoma to central Texas (Fig. V.2A). *Baccharis halmifolia* core habitat occupies most of the southeastern US from East Texas north along the coast to Rhode Island (Fig. V.2B). Much of the southwestern US and northern Mexico is occupied by core habitat of *B. salicina* (Fig. V.3A). Core habitat of *S. altissima* comprises most of the eastern US, including most of Oklahoma (Fig. V.3B). The entire

Modified CEC Level III Ecoregions

Arkansas Valley (1)	Middle Central Great Plains (8)	Southern Central Irregular Plains (15)
Boston Mountains (2)	Mississippi Alluvial Plain (9)	Southern High Plains (16)
Cross Timbers (3)	Mississippi Valley Loess Plains (10)	Southern Texas Plains (17)
East Central Texas Plains (4)	Ouachita Mountains (11)	Texas Blackland Prairies (18)
Eastern Edwards Plateau (5)	Ozark Highlands (12)	Western Edwards Plateau (19)
Eastern Southwestern Tablelands (6)	South Central Plains (13)	Western Gulf Coastal Plain (20)
Flint Hills (7)	Southern Central Great Plains (14)	



2011 National Land Cover Database

Barren Land	Developed, Medium Intensity	Mixed Forest
Cultivated Crops	Developed, Open Space	Open Water
Deciduous Forest	Emergent Herbaceous Wetlands	Pasture/Hay
Developed, High Intensity	Evergreen Forest	Shrub/Scrub
Developed, Low Intensity	Grassland/Herbaceous	Woody Wetlands

Fig. V.1. South-Central US study area for land cover risk assessment analyses with land cover classes of the 2011 National Land Cover Database (Homer et al. 2015, MLRC 2017) and modified CEC (2005) Level III ecoregions. Ecoregions were modified to delete portions non-contiguous with the study area or in Mexico, and add regional divisions for several Level III ecoregions (for details, see Appendix E Methods).

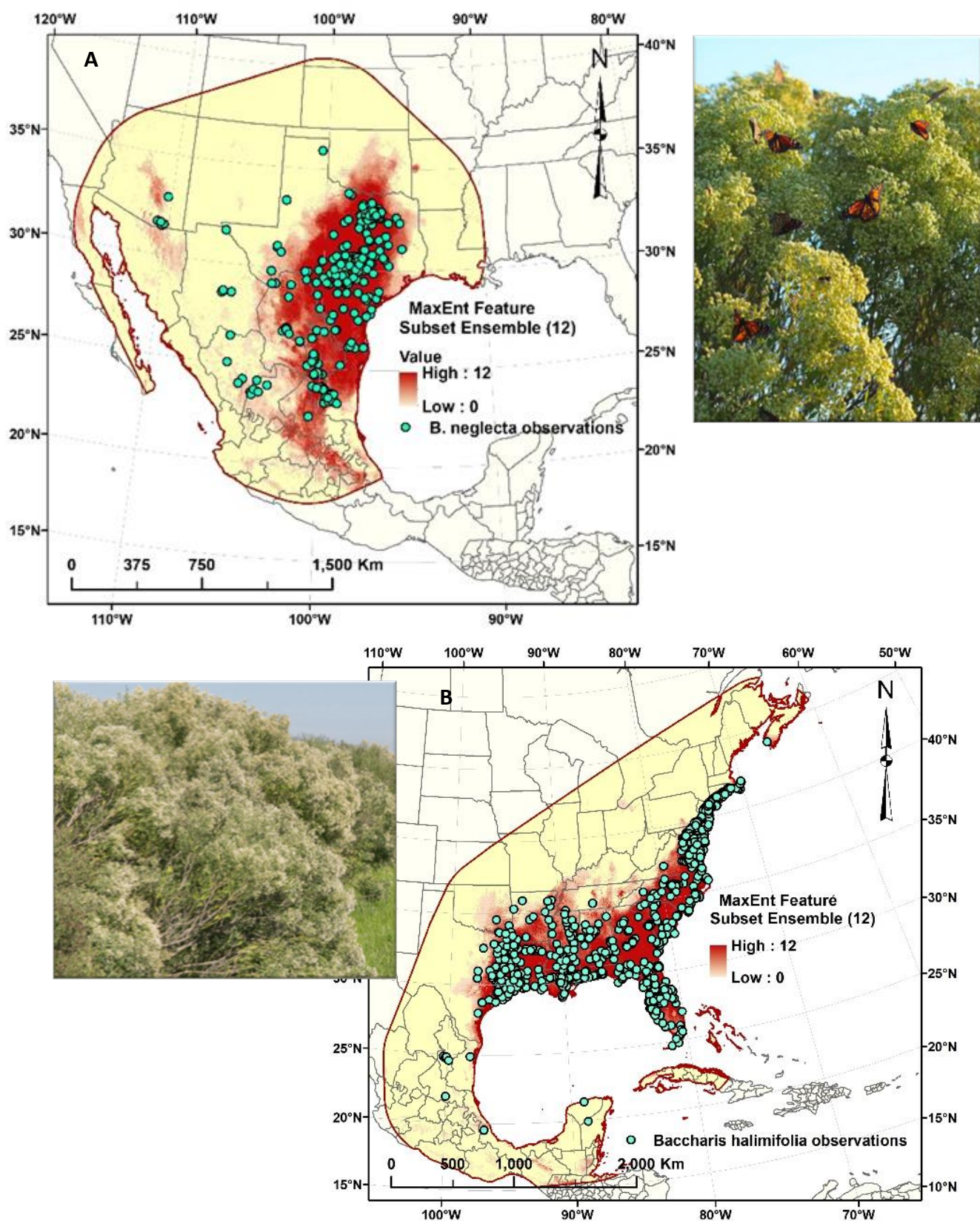


Fig. V.2. (A) *Baccharis neglecta* and (B) *B. halimifolia* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

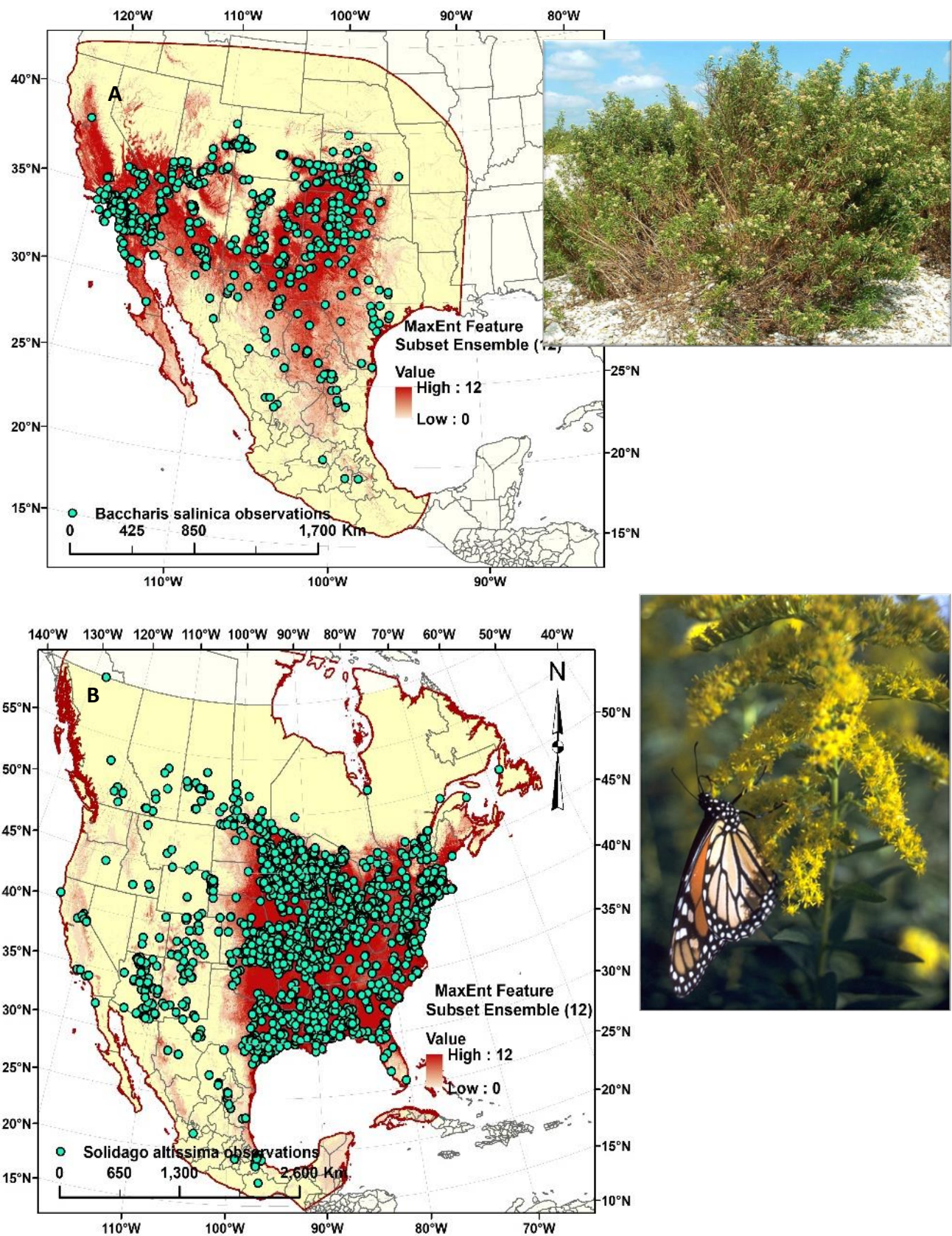


Fig. V.3. (A) *Baccharis salicina* and (B) *Solidago altissima* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

southeastern US comprises the core habitats of both *Verbesina virginica* and *Conoclinium coelestinum* (Fig. V.4A,B). The core distribution of *Viguiera stenoloba* extends from southeastern New Mexico through southwestern Texas and covers much of northeastern Mexico (Fig. V.5A). *Verbesina enceliodes* core habitat occupies much of the southwestern US, including most of Texas and the western two thirds of Oklahoma (Fig. V.5B.). The core distribution of *Glandularia bipinnatifida* covers most of Texas, excluding the Trans Pecos and the most southern regions, and extends north over most of Oklahoma and Kansas (Fig. V.6A). Most of the southeastern coastal states comprise the core distribution of *Verbena halei*, with the exception of northwestern and Trans Pecos Texas and southern Florida (Fig. V.6B).

Significant differences were found among most NLCD land cover class risk values, as indicated LCUI, for each of the ten nectar species (Tables V.2-3). Among the South-Central US MaxEnt core habitats for the three *Baccharis* species, *B. neglecta* had the highest land cover areas of high risk (low LCUI), consisting of Cultivated Crops and Pasture/Hay NLCD land cover classes in the Texas Blackland Prairies and southern parts of the Western Gulf Coastal Plain (Figs. V.7-9). High risk core habitat for *Solidago altissima* consisted of Cultivated Croplands concentrated along the Mississippi Alluvial Plain of Louisiana (Fig. V.10). The South-Central US MaxEnt core habitats for *Verbesina enceliodes* and *Conoclinium coelestinum* are similar but their high risk land covers are distinctive. Areas of high risk land cover for *V. enceliodes* are more evident within the Texas Blackland Prairies and the eastern edge of the Western Gulf Coastal Plain. Areas of high risk land covers for *C. coelestinum*, such as Cultivated Crops and Pasture/Hay, are most abundant in the southeastern range within the central Western Gulf Coastal Plain and East Central Texas Plains. (Figs. V.11-12). The core habitat of *Viguiera stenoloba* was limited to the eastern edge of the South-Central US and exhibited little area impacted by high risk land covers, such as Cultivated Crops (Fig. V.13). Core habitat of *Verbesina enceliodes* occupied much of the western edge of the South-Central US study area in Texas, with Cultivated Crops representing moderate risk within the Southern High Plains of the panhandle and the southern tip of the Western Gulf Coastal Plain (Fig. V.14). Both Pasture/Hay and Cultivated Crops represent large areas of high risk land cover for *Glandularia bipinnatifida* in the eastern portion of its range in Texas, along the Western Gulf Coast Plains, East Central Texas Plains, and Texas Blackland Prairies (Fig. V.15). High risk Cultivated Crops land cover for *Verbena halei* core habitat in Texas occurred in large patches of the Western Gulf Coastal Plain and Southern Central Great Plains (Fig. V.16).

Discussion

The core distributions of all ten nectar species covered widely varying portions of the South-Central US study areas over both the fall and spring flowering seasons. The Cultivated Crops land cover represent high land cover risk (low LCUI) for seven of the ten nectar plant species in the South-Central US. Areas with highest Cultivated Crops for nectar species in the study area include the Texas Blackland Prairies, East Central Texas Plains, Western Coastal Gulf Plain, and Mississippi Alluvial Plain. Four of the ten nectar species were found to have high land cover risk from Pasture/Hay areas, mostly in the East Central Texas Plains. Developed, Low Intensity land cover, representing roadsides and urban areas, was the most low risk favorable land cover for six of the ten nectar plant species. However, such habitats may be unavailable during parts of the spring or fall monarch migration due to mowing. Milkweeds (*Asclepias* sp.),

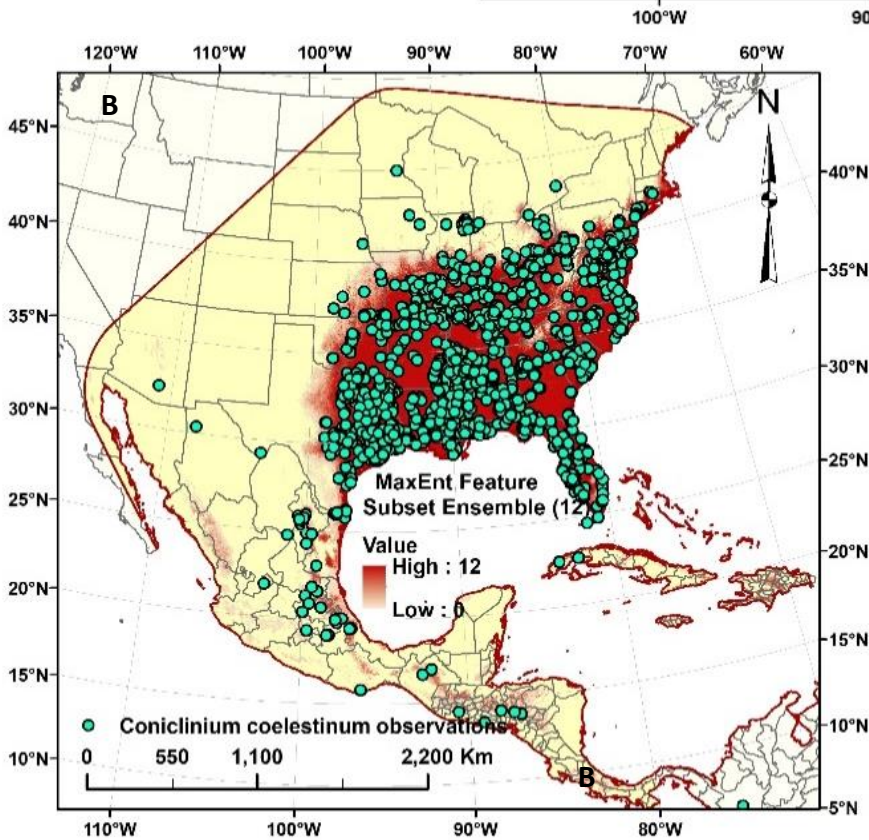
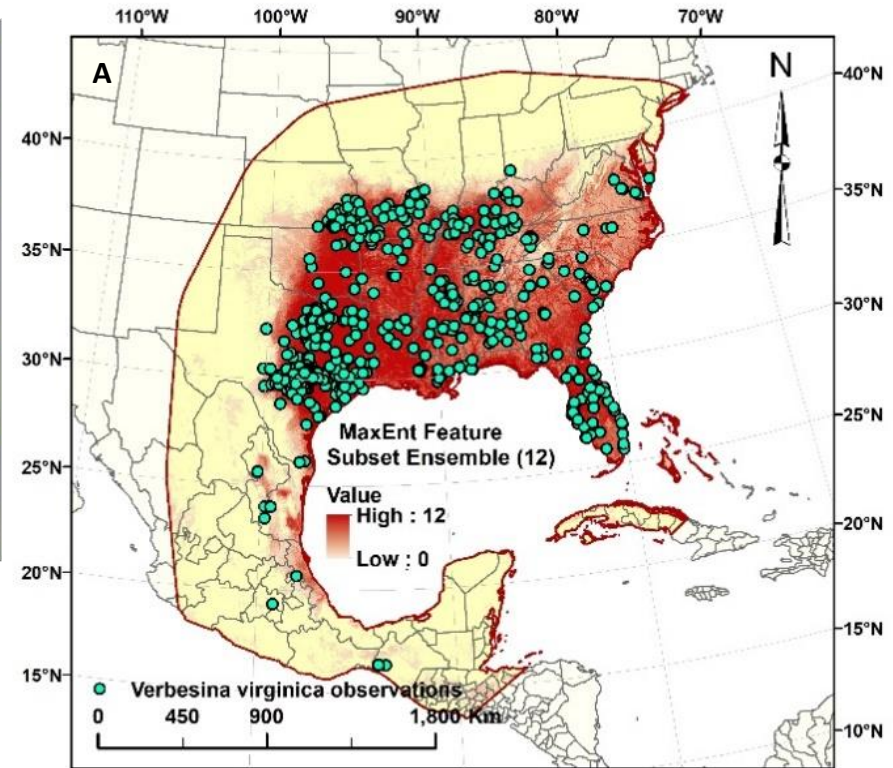


Fig. V.4. (A) *Verbescina virginica* and (B) *Conoclinium coelestinum* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

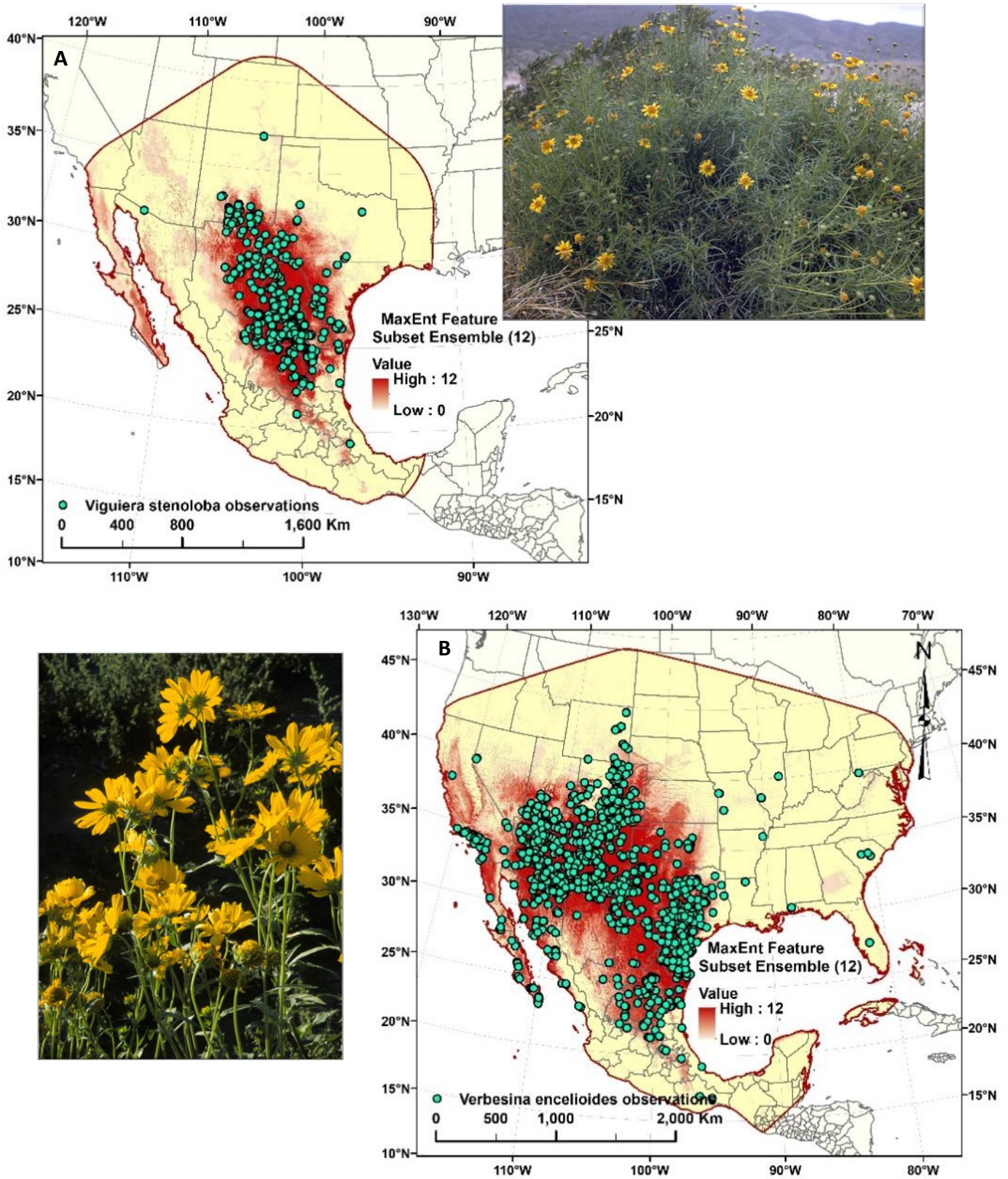


Fig. V.5. (A) *Viguiera stenoloba* and (B) *Verbesina encelioides* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

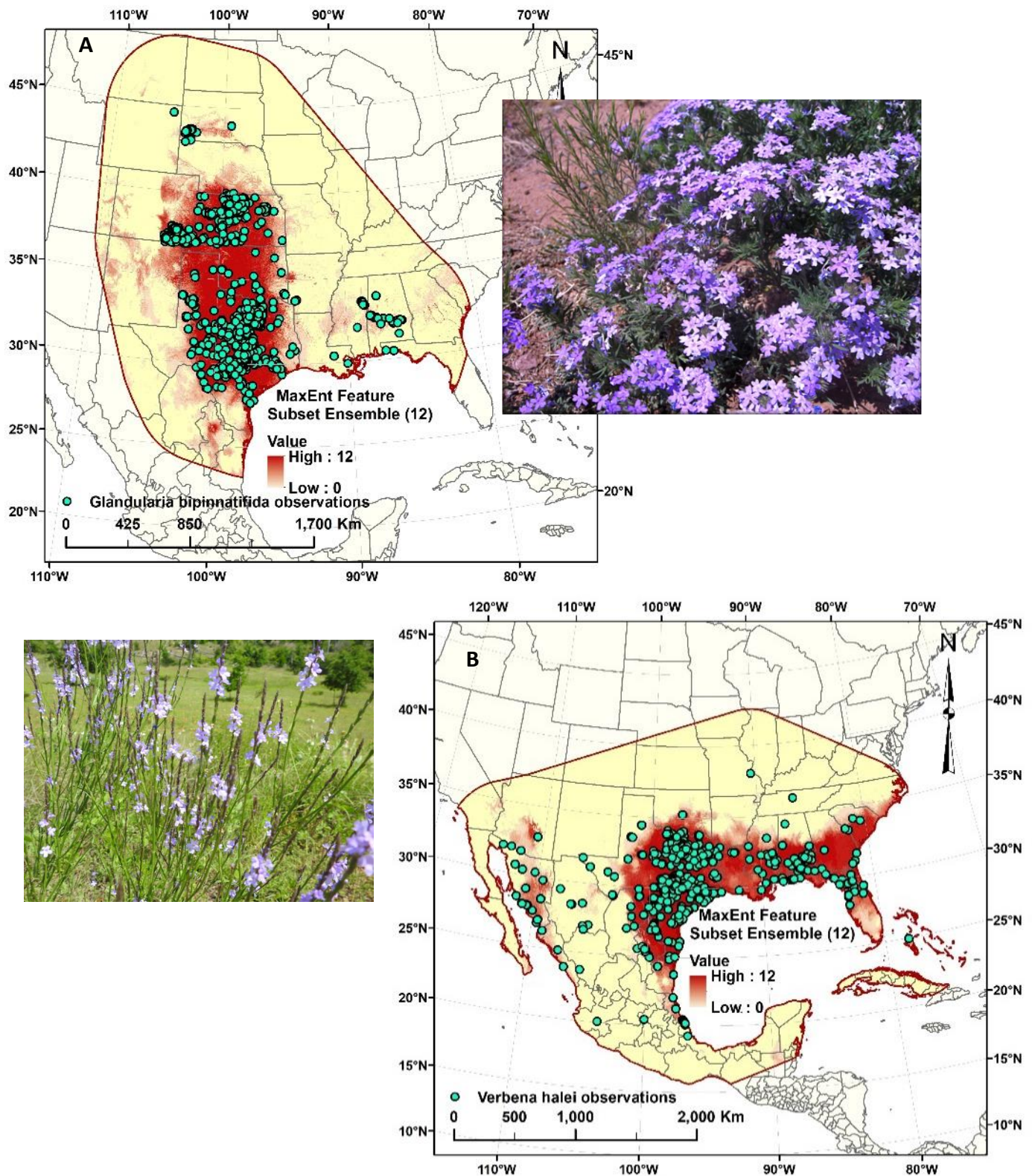


Fig. V.6. (A) *Glandularia bipinnatifida* and (B) *Verbena halei* MaxEnt frequency consensus for feature subset ensemble of 12 models developed from random subsets of 95 variables, including MaxEnt 100% consensus core habitat and CEC (2005) ecoregions.

Table V.2.. Mean land cover utilization index (LCUI) over 12 MaxEnt modeled core habitats in South-Central US study area for five nectar plant species.

2011 National Land Cover Database (NLCD) Land Cover Class	Mean Land Cover Utilization Index (%; n = 12; \pm SD) ^a				
	<i>Baccharis neglecta</i>	<i>Baccharis halimifolia</i>	<i>Baccharis salicina</i>	<i>Solidago altissima</i>	<i>Verbesina virginica</i>
Cultivated Crops	6.4 \pm 0.4h*	55.9 \pm 12.6g*	67.1 \pm 7.9d*	25.1 \pm 1.1i*	3.1 \pm 0.2k*
Deciduous Forest	154.5 \pm 7.8bc*	32.9 \pm 2.3f*	2.7 \pm 0.7f*	47.6 \pm 2.4g*	105.2 \pm 3.0d*
Developed, Low Intensity	245.8 \pm 7.0a*	268.5 \pm 12.9a*	106.3 \pm 17.6c	463.8 \pm 20.6a*	56.8 \pm 1.9g*
Developed, Medium Intensity	NA	NA	NA	NA	NA
Developed, Open Space	147.8 \pm 2.8c*	233.7 \pm 8.2b*	26.5 \pm 1.0e*	198.7 \pm 1.9d*	205.7 \pm 2.8b*
Emergent Herb. Wetlands	NA	231.1 \pm 27.0b*	NA	281.2 \pm 44.9b*	52.6 \pm 14.0h*
Evergreen Forest	117.8 \pm 6.9d*	72.5 \pm 4.5f*	32.2 \pm 9.3e*	59.9 \pm 1.6f*	235.8 \pm 13.6a*
Grassland Herbaceous	56.5 \pm 2.4e*	49.7 \pm 9.3g*	99.6 \pm 9.8c	73.0 \pm 5.2e*	89.5 \pm 3.4e*
Mixed Forest	63.3 \pm 15.9e*	28.3 \pm 2.3h*	NA	241.5 \pm 13.6c*	33.1 \pm 1.9i*
Pasture/Hay	22.5 \pm 1.3f*	90.0 \pm 5.4e*	77.4 \pm 26.4cd*	72.5 \pm 4.3e*	23.1 \pm 1.0j*
Shrub/Scrub	157.6 \pm 4.2b*	126.0 \pm 9.3c*	139.4 \pm 12.6b*	256.4 \pm 52.6bc*	180.4 \pm 13.4c*
Woody Wetlands	15.3 \pm 1.6g*	101.5 \pm 11.5d	519.9 \pm 87.1a*	43.6 \pm 2.6h*	67.2 \pm 4.4f*

^an = 12 MaxEnt model areas of overlap with 100% consensus core habitat MaxEnt model. Means within a replicate with a different letter are significantly different ($P < 0.05$; Kruskal-Wallis Rank Sum Test followed by Pairwise Wilcoxon Rank Sum Test). Means with asterisks signify Land Cover Value Index (LCVI = LCUI – 100) for class is significantly different from zero, indicating land cover utilization is different from expected (LCUI = 100) ($P < 0.05$; Paired Wilcoxon Signed Rank Test).

Table V.3. Mean land cover utilization index (LCUI) over 12 MaxEnt modeled core habitats in South-Central US study area for five nectar plant species.

2011 National Land Cover Database (NLCD) Land Cover Class	Mean Land Cover Utilization Index (%; n = 12; \pm SD) ^a				
	<i>Conoclinium coelestinum</i>	<i>Viguiera stenoloba</i>	<i>Verbesina encelioides</i>	<i>Glandularia bipinnatifida</i>	<i>Verbena halei</i>
Cultivated Crops	5.4 \pm 0.3i*	0.8 \pm 0.2f*	62.4 \pm 3.8f*	10.0 \pm 0.7j*	24.0 \pm 2.3i*
Deciduous Forest	108.4 \pm 5.8d*	3.2 \pm 2.6e*	118.5 \pm 19.9c*	71.1 \pm 5.1f*	68.7 \pm 3.3f*
Developed, Low Intensity	113.7 \pm 6.5d*	84.5 \pm 12.7c*	258.0 \pm 18.9a*	378.5 \pm 15.4a*	438.8 \pm 23.0a*
Developed, Medium Intensity	NA	66.6 \pm 16.8d*	NA	NA	NA
Developed, Open Space	192.8 \pm 1.5b*	138.8 \pm 17.1b*	232.4 \pm 3.8b*	328.3 \pm 3.1b*	324.4 \pm 10.9b*
Emergent Herb. Wetlands	69.6 \pm 5.2f*	NA	NA	12.7 \pm 3.8i*	90.1 \pm 23.3e*
Evergreen Forest	72.5 \pm 3.1f*	NA	97.1 \pm 6.4d	176.4 \pm 21.5c*	63.8 \pm 6.8g*
Grassland Herbaceous	47.3 \pm 4.3g*	134.7 \pm 18.9b*	104.9 \pm 6.7c*	81.2 \pm 2.5e*	109.1 \pm 4.0c*
Mixed Forest	140.9 \pm 10.7c*	NA	32.7 \pm 10.2g*	40.9 \pm 9.4g*	58.6 \pm 6.5h*
Pasture/Hay	32.1 \pm 1.9h*	455.7 \pm 128.7a*	86.0 \pm 8.9e*	25.1 \pm 1.2h*	70.2 \pm 4.2f*
Shrub/Scrub	395.6 \pm 76.3a*	88.2 \pm 6.4c*	88.4 \pm 6.4e*	149.0 \pm 3.0d*	98.6 \pm 6.7d
Woody Wetlands	89.8 \pm 7.1e*	3.6 \pm 0.8e*	226.5 \pm 37.8b*	8.5 \pm 1.6k*	91.7 \pm 10.2e*

^an = 12 MaxEnt model areas of overlap with 100% consensus core habitat MaxEnt model. Means within a replicate with a different letter are significantly different ($P < 0.05$; Kruskal-Wallis Rank Sum Test followed by Pairwise Wilcoxon Rank Sum Test). Means with asterisks signify Land Cover Value Index (LCVI = LCUI – 100) for class is significantly different from zero, indicating land cover utilization is different from expected (LCUI = 100) ($P < 0.05$; Paired Wilcoxon Signed Rank Test).

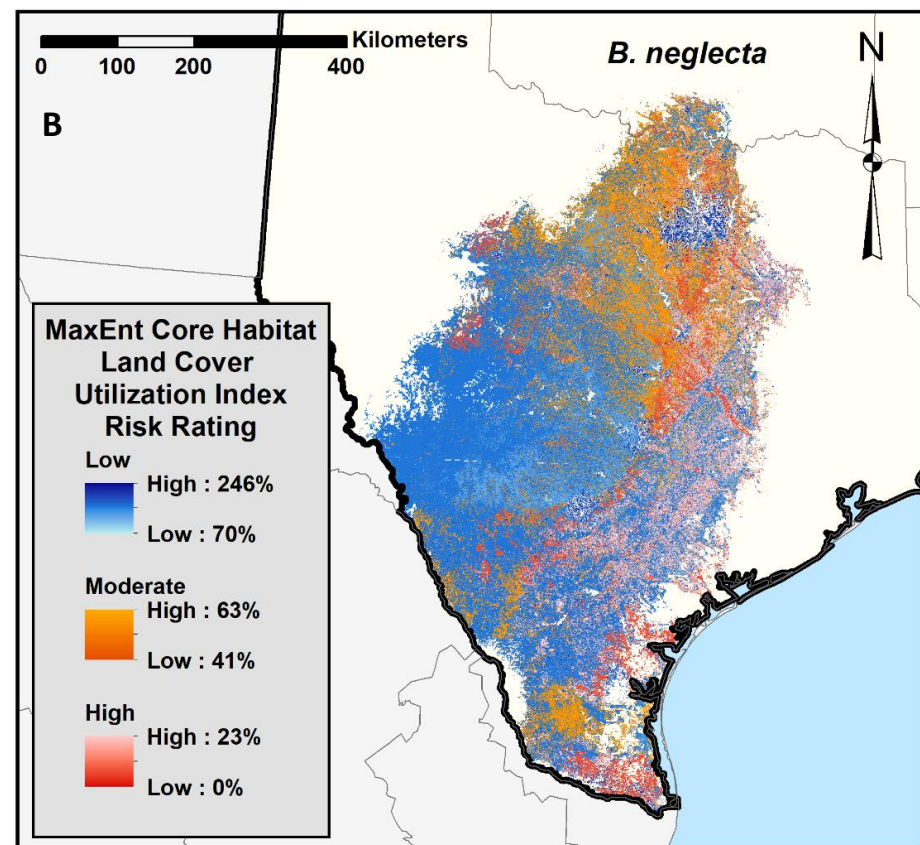
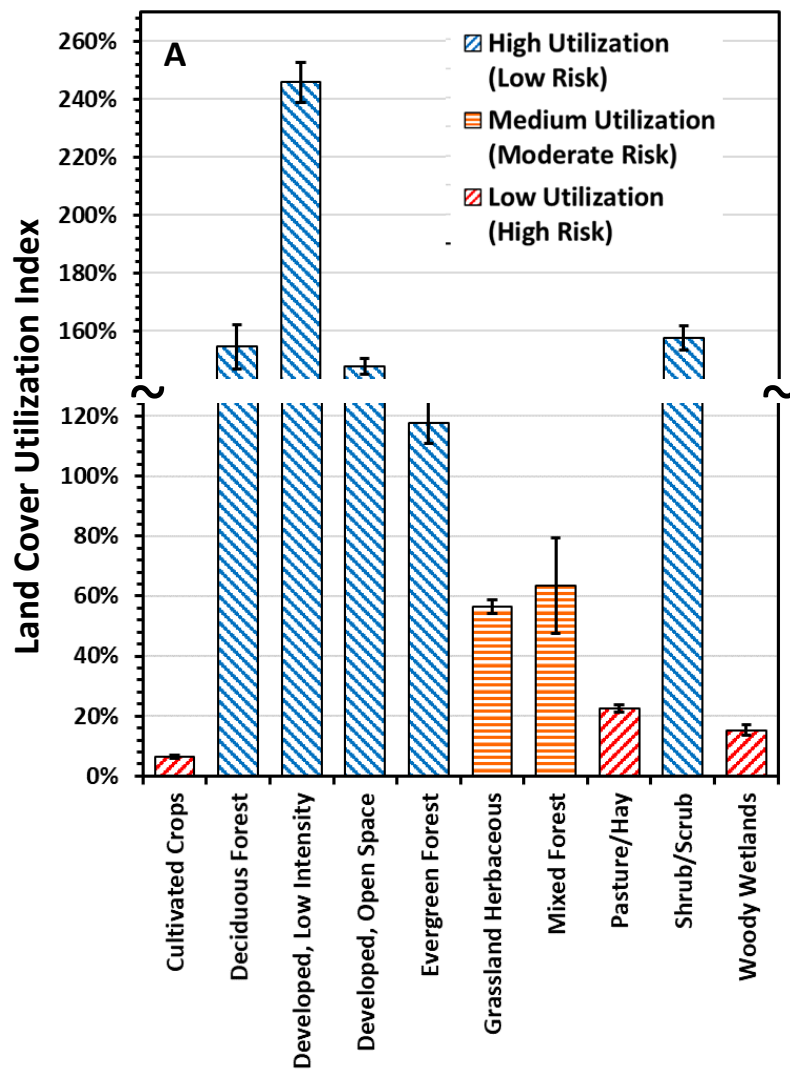


Figure V.7. *Baccharis neglecta* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

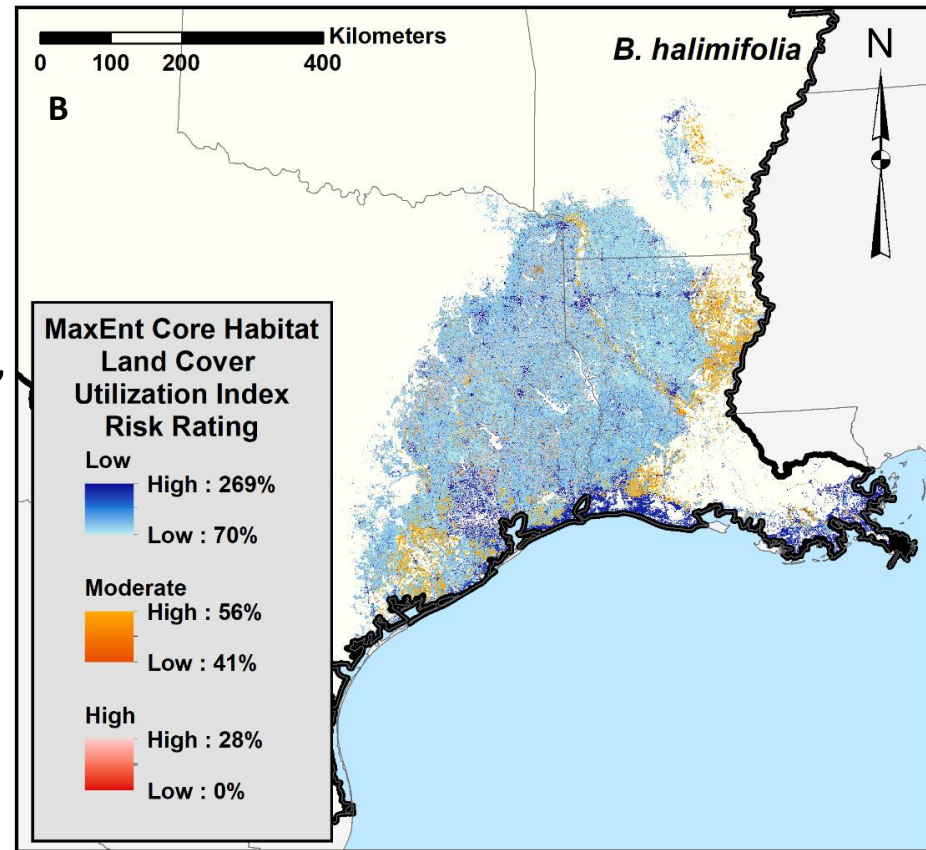
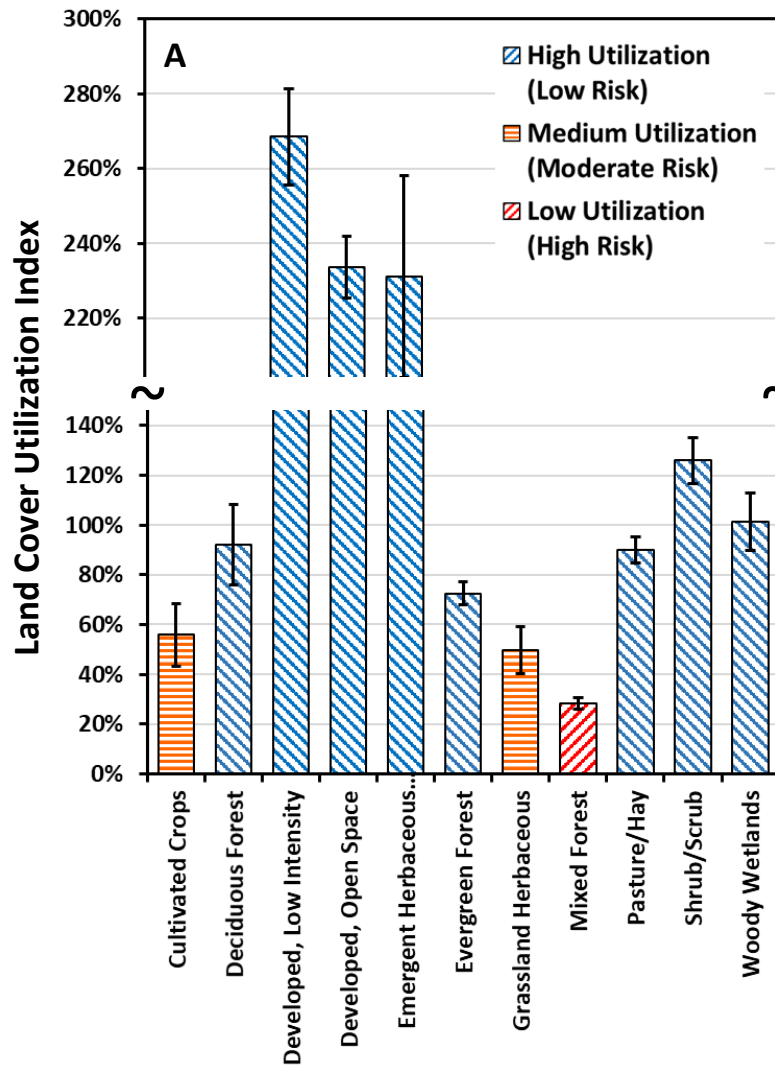


Figure V.8. *Baccharis halimifolia* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

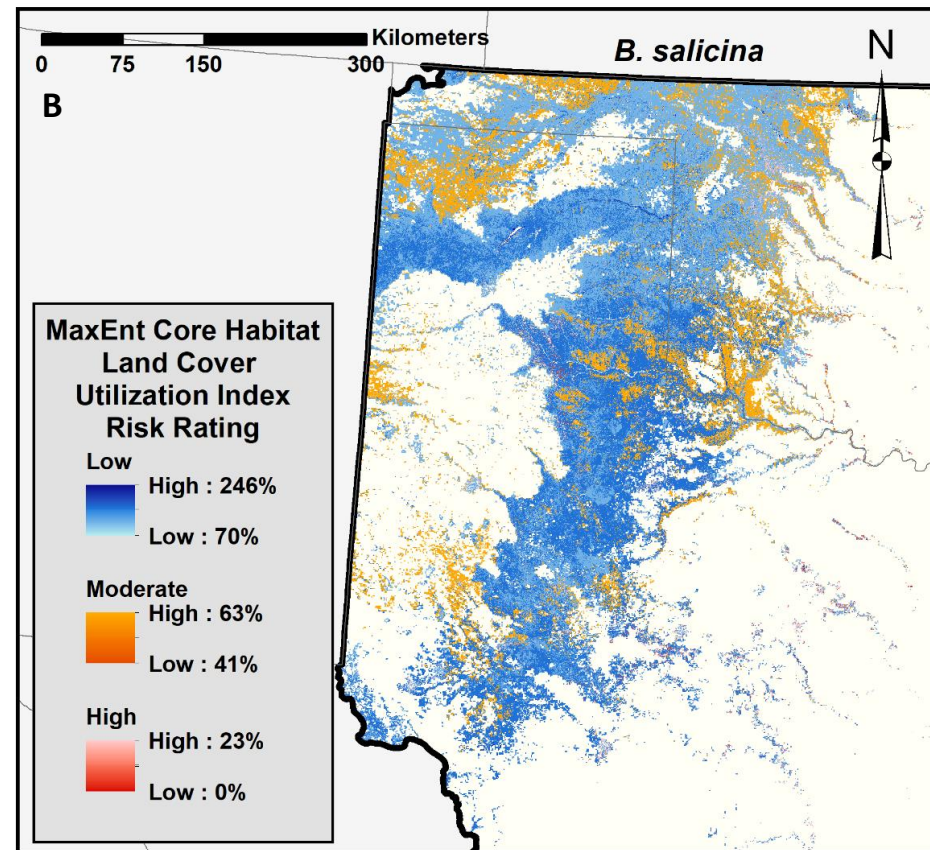
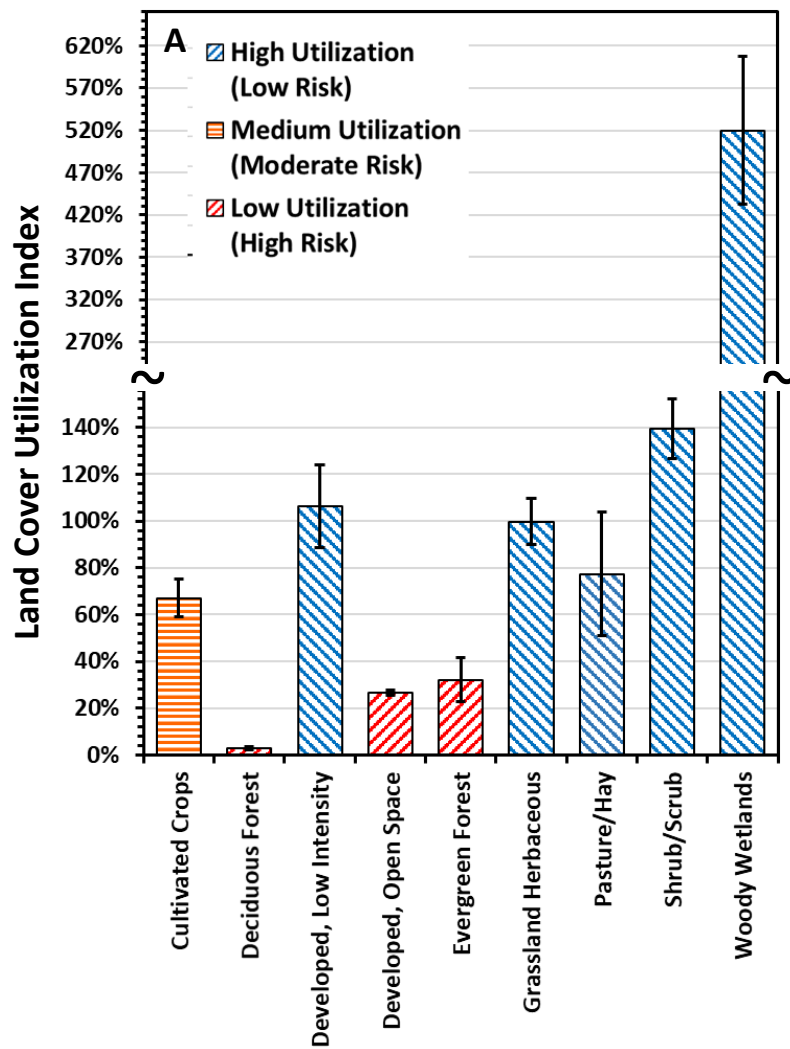


Figure V.9. *Baccharis salicina* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

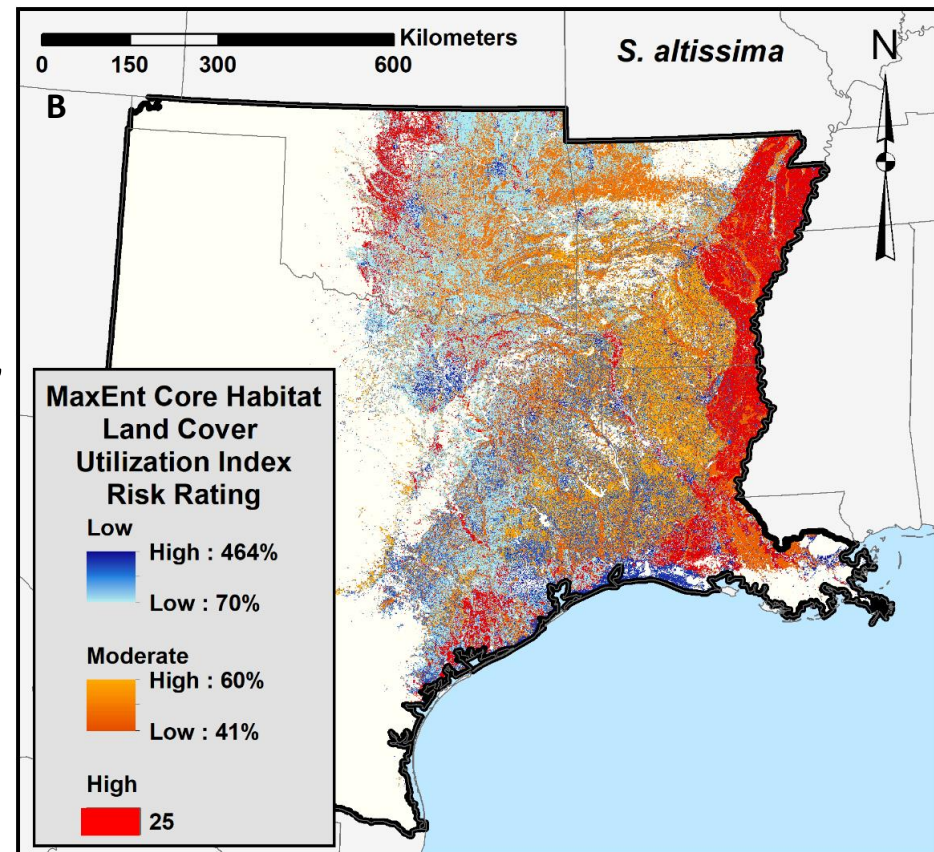
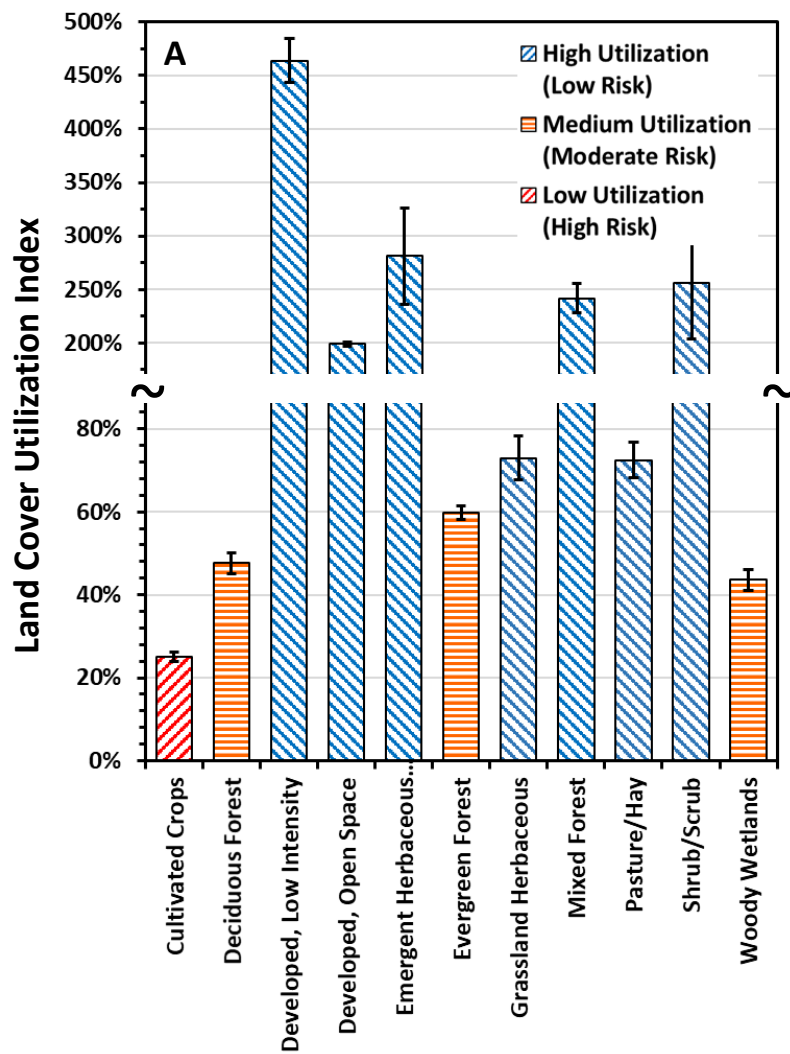


Figure V.10. *Solidago altissima* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

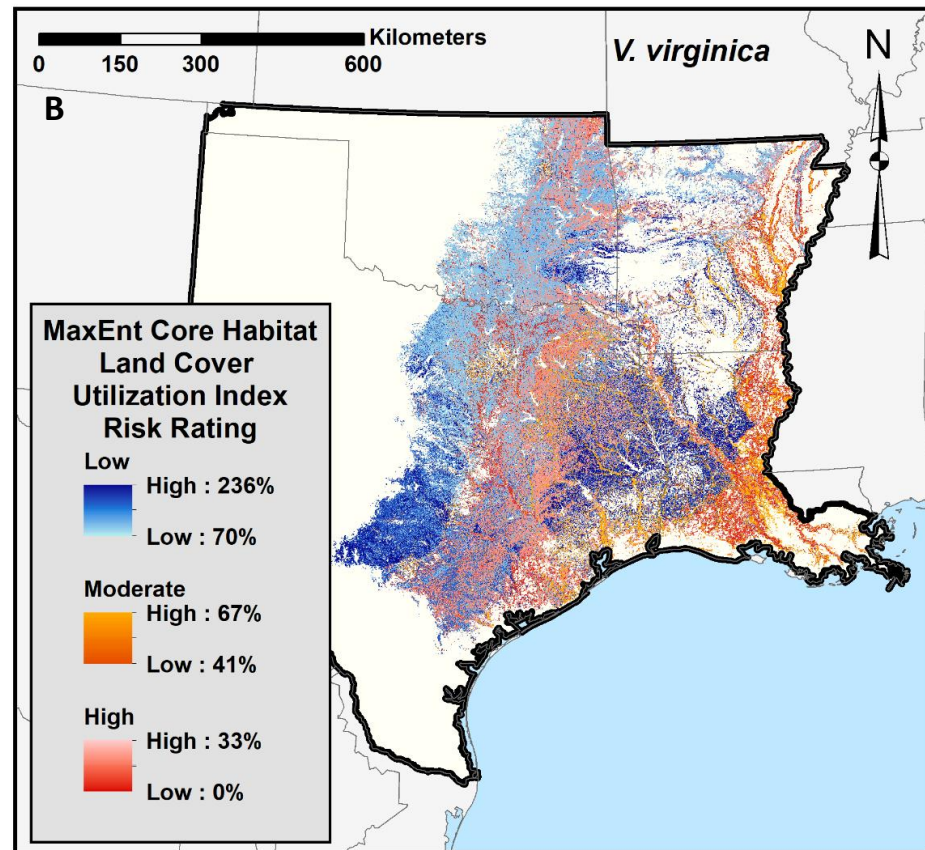
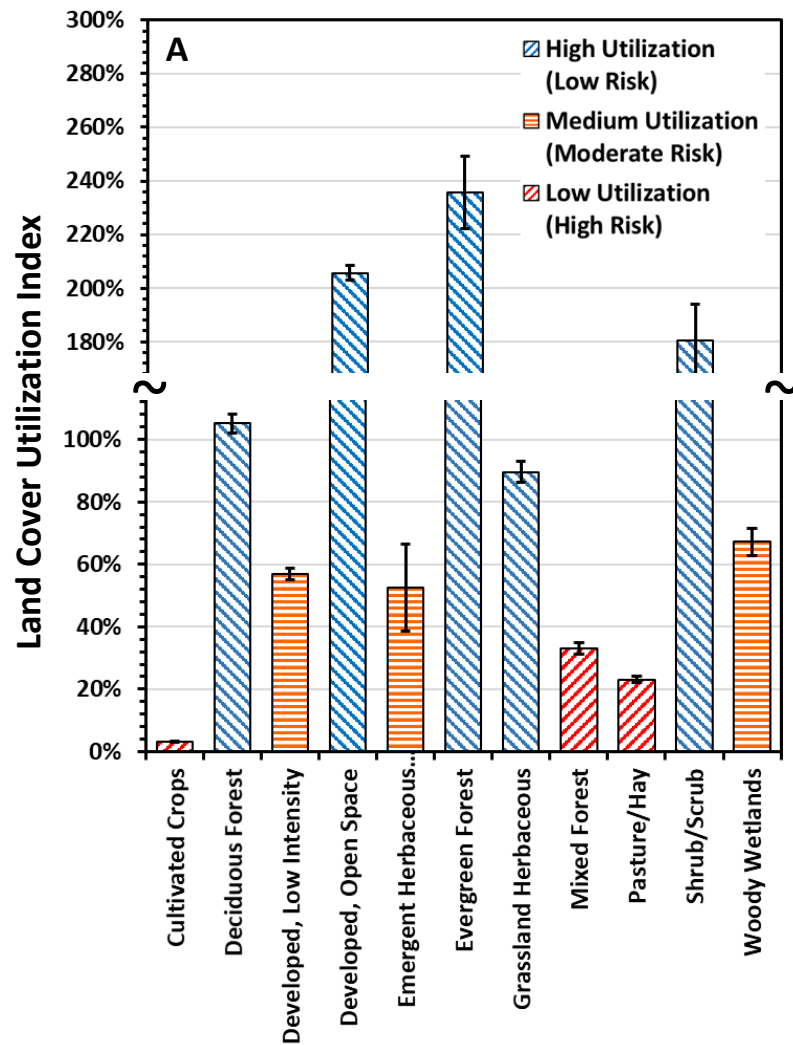


Figure V.11. *Verbesina virginica* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

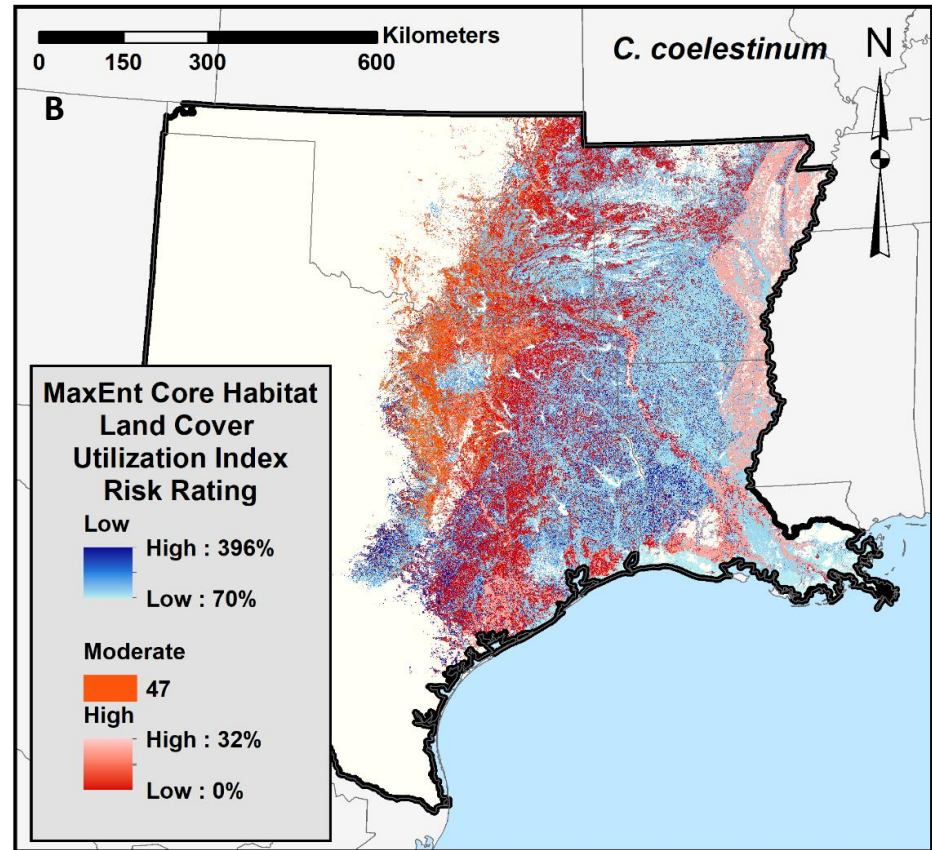
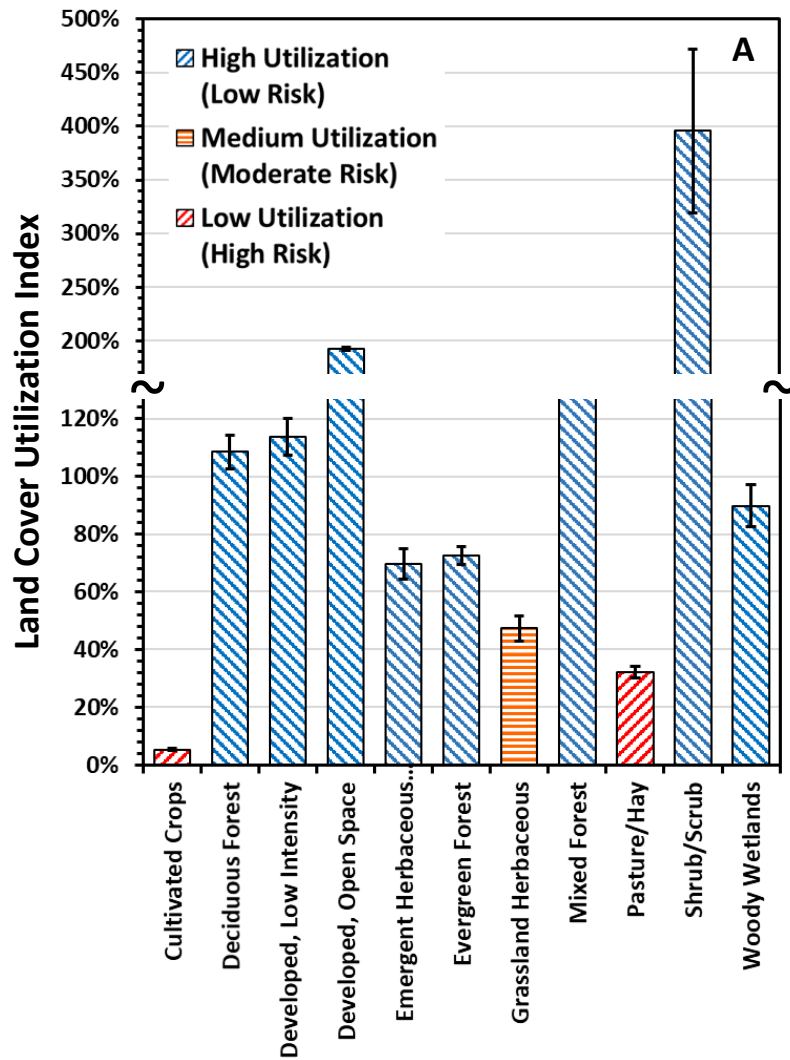


Figure V.12. *Conoclinium coelestinum* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

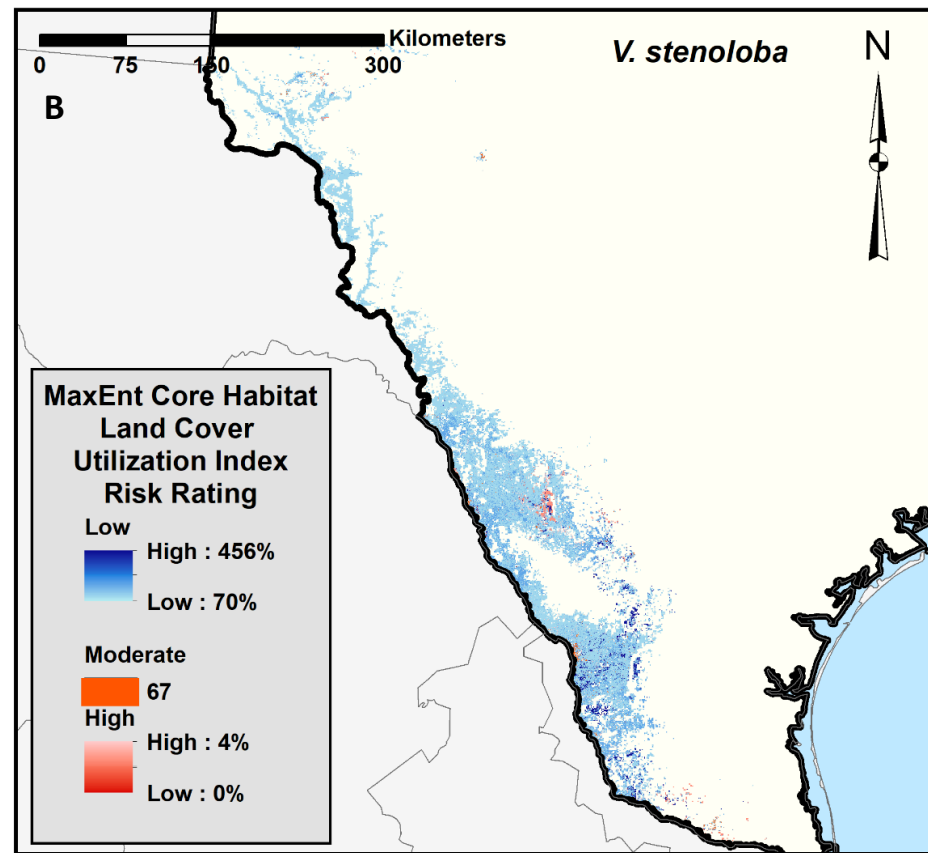
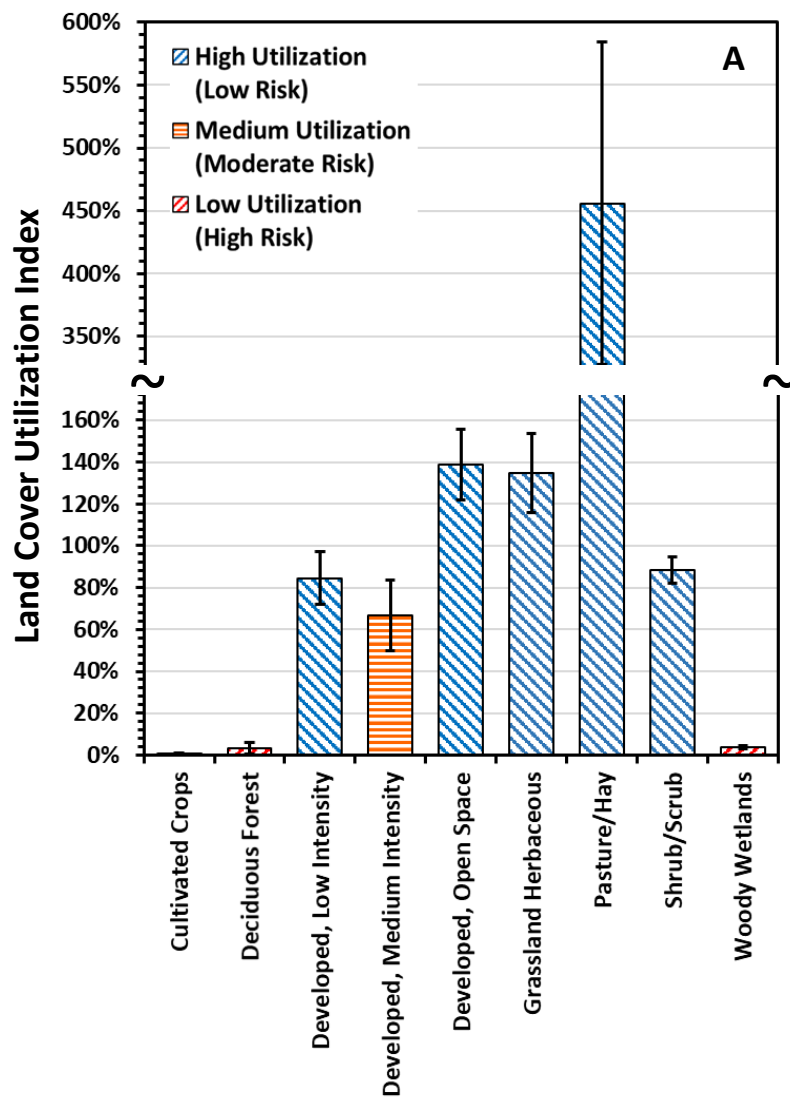


Figure V.13. *Viguiera stenoloba* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

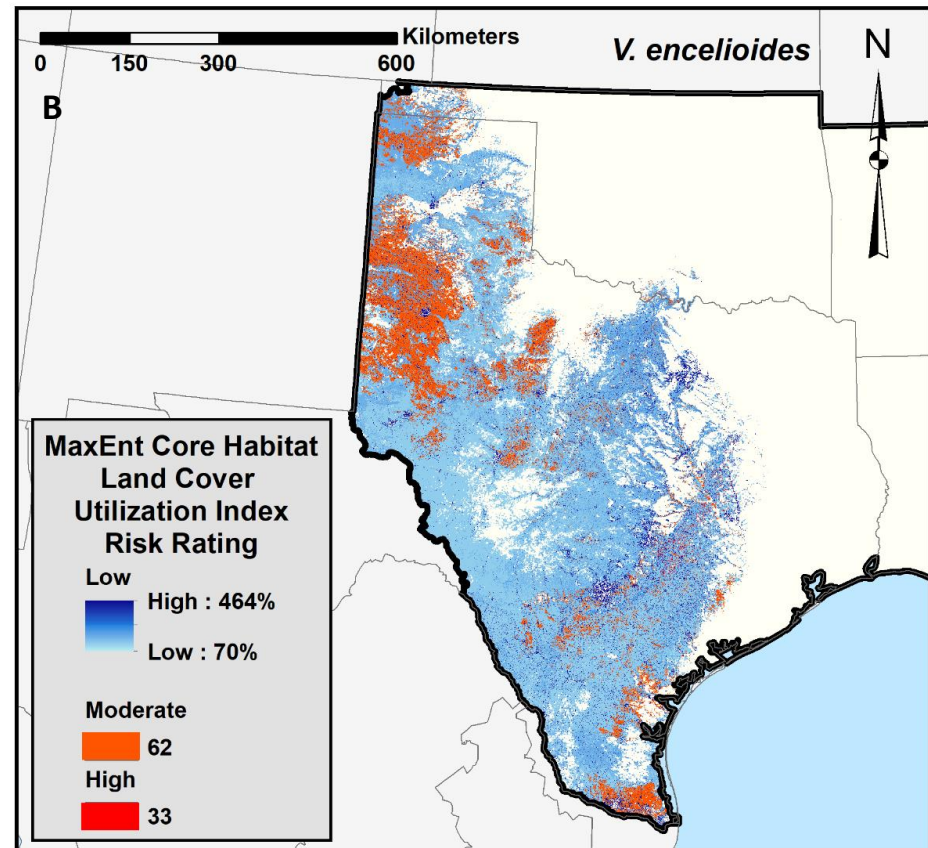
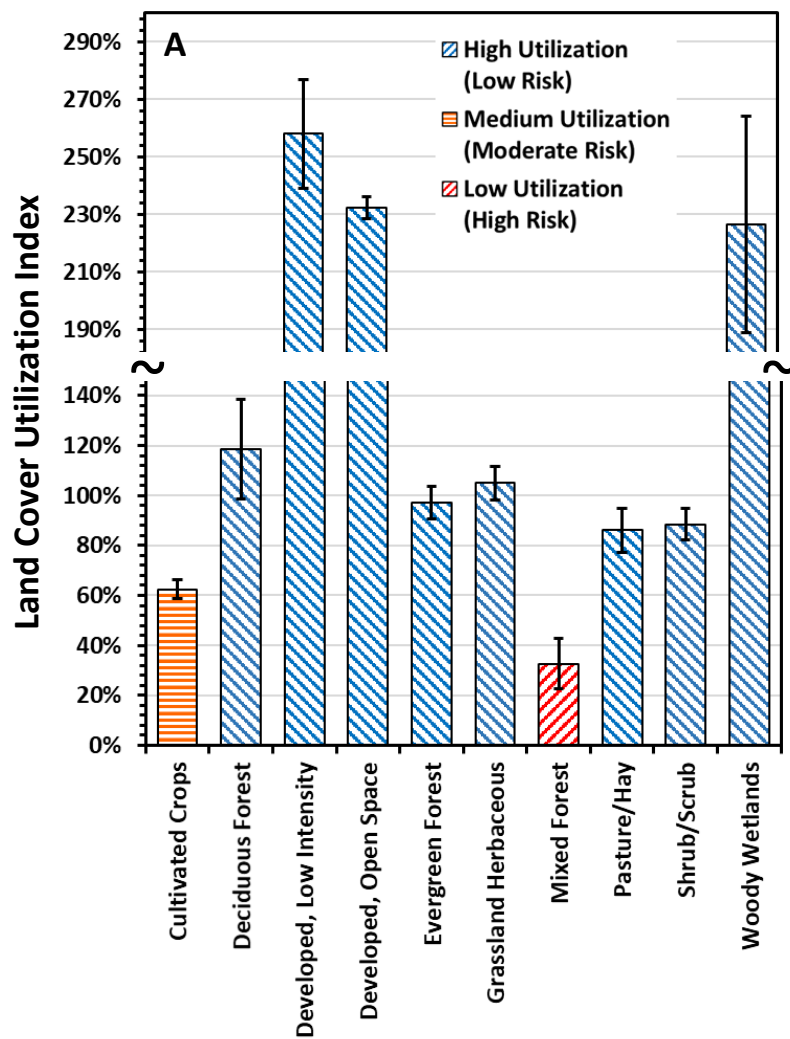


Figure V.14. *Verbesina encelioides* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

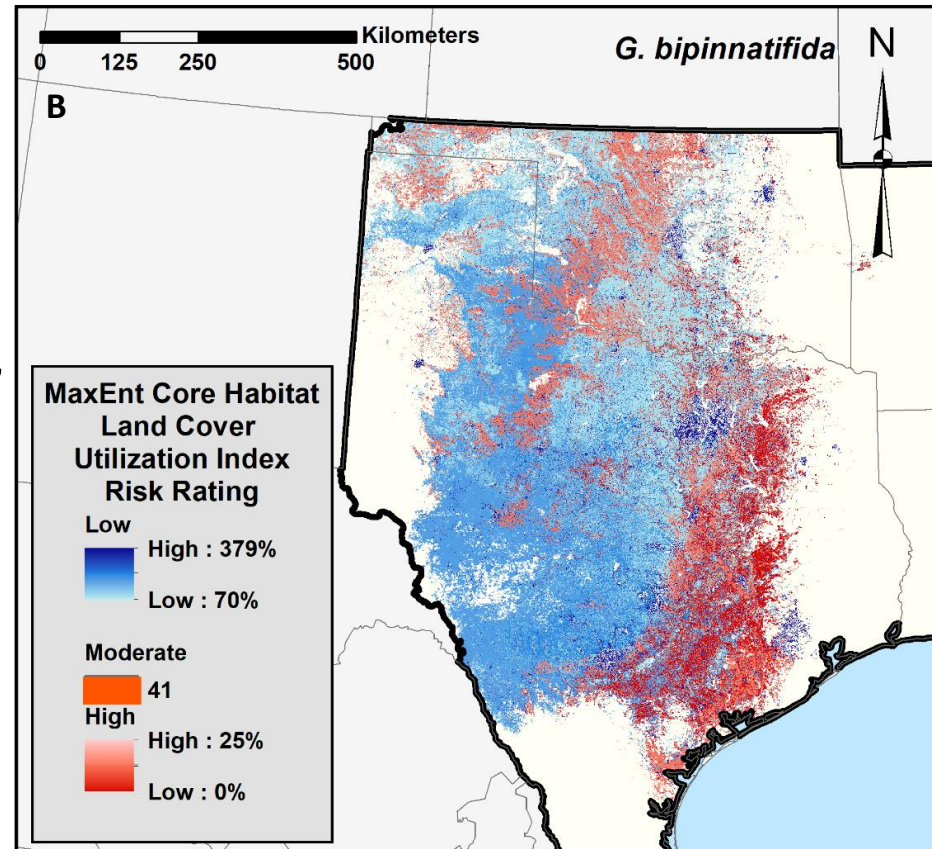
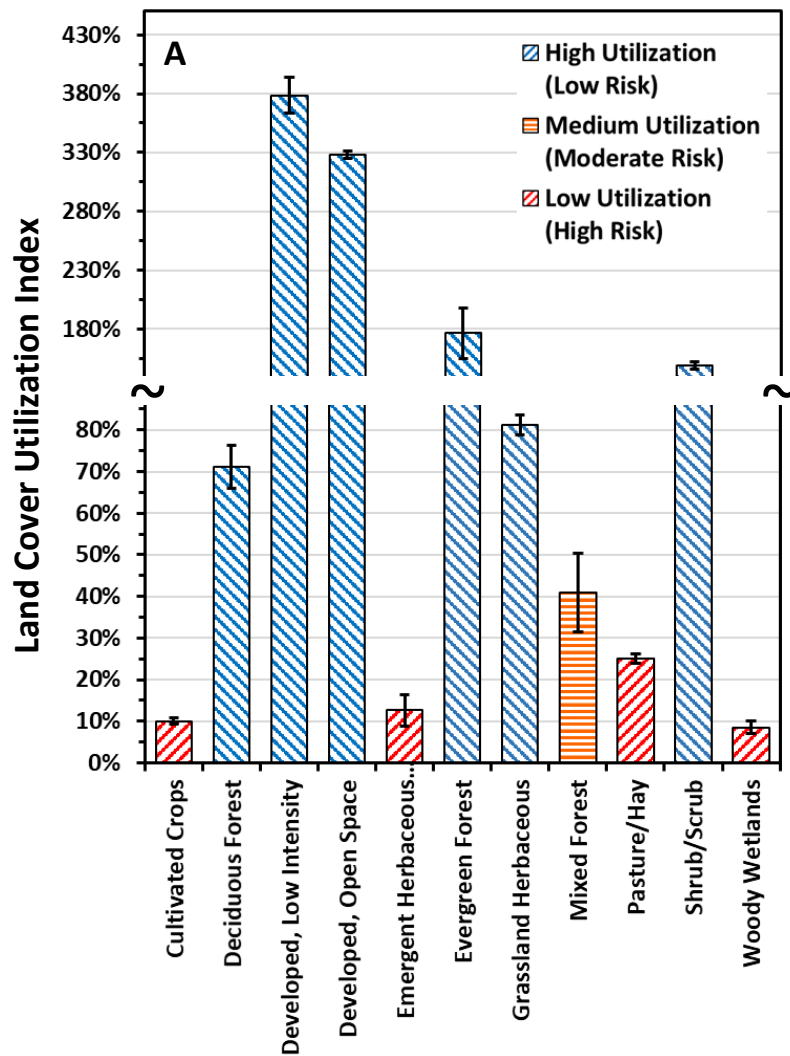


Figure V.15. *Glandularia bipinnatifida* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

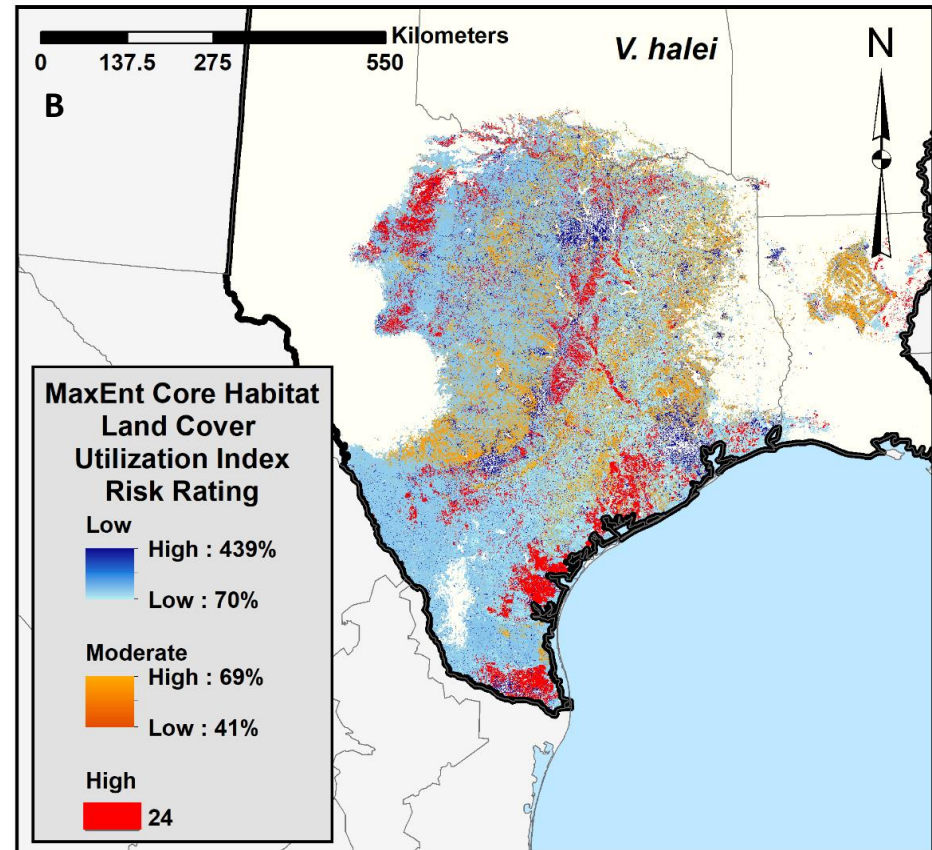
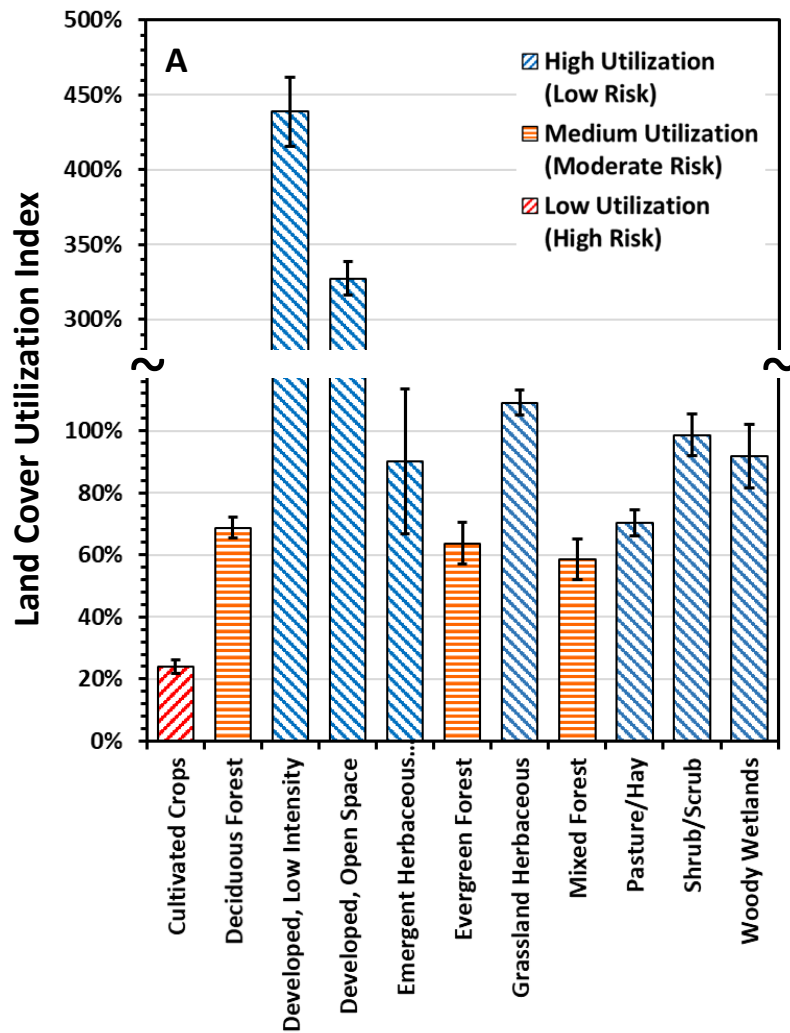


Figure V.16. *Verbena halei* (A) land cover utilization index (LCUI) risk ratings for various 2011 National Land Cover Dataset (NLCD) land cover classes over MaxEnt core habitat for South-Central US region, and (B) distribution of high and low LCUI risk areas over the South-Central region core habitat.

which are the monarch larval host plants, are also excellent sources of nectar for the adults (USDA NRCS 2015a,b) and are subject to the same risks from Cultivated Crop land cover and mowing of roadsides (see Section III).

The USDA NRCS Monarch Butterfly Habitat Development Project (USDA NRCS 2015) includes providing landowner incentives for increasing monarch nectar plants in the southern Great Plains, including Oklahoma and Texas. Strategies for increasing nectar plants include disturbance from prescribed burning and light disking. Additional targeting of conservation actions to increase nectar plants can be focused in identified ecoregions with high Cultivated Crop and Pasture/Hay land covers. In addition, guidelines for avoiding mowing of roadsides during the spring and fall migrations (Monarch Joint Venture 2016) can help preserve the many monarch nectar source species abundant in these areas.

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SECTION VI: MONARCH WEEKLY PRE-MIGRANT/MIGRANT MODELS

Introduction

The main mass of monarch migrants generally begins to reach north central Texas around 29 September (Calvert 1999, Monarch Watch 2017, Journey North 2017). Immature monarchs present in the South-Central US (Texas, Oklahoma, Louisiana and Arkansas) from September and through early November are considered to be part of the fifth generation that is produced by pre-migrant monarchs. Pre-migrants are also referred to as late or fall breeding monarchs that move south early, prior to migration, and reproduce in Texas and surrounding states (Calvert 1999, Prysby and Oberhauser 2004, Baum and Sharber 2012, Mueller and Baum 2014, Batalden and Oberhauser 2015). Borland et al. (2004) examined the proportion of mated females collected throughout Texas from mid-September to late October. They found that about 60-80% of pre-migrants collected around 18-21 September were reproductive, compared to generally less than 40% of migrants being reproductive in late October. It is currently not known what proportion of the overall population is comprised of pre-migrants, or how important this fifth generation of monarchs is to the overall monarch population (but see Flockhart et al. 2017). It is possible that the importance of this fifth generation has changed with milkweed loss in the Upper Midwest or that the importance varies among years.

Calvert (1999) inventoried milkweed and monarch egg and larva along an east to west transect in Texas from Ozona through Austin to Pineville between 21 September and 18 November 1996. Above average fall precipitation likely contributed to the presence of *A. viridis*, *A. a. capricornu*, *A. oenotheroides*, and *A. latifolia* along various parts of the transect. He found eggs (including those of the queen butterfly, *Danaus gilippus*) and fifth generation monarch larvae originating from pre-migrants on all of these milkweed species along the eastern and western ends of the transect. The number of eggs and larvae declined in the western and eastern ends of the transect as the season progressed. Both eggs and larvae were found east of Austin in late September but not in early October or November. However, eggs and larvae were found west of Austin also from September 27 to 17 November. He suggested that the monarch breeding was already over in East Texas by the time the main migration mass arrived, but simultaneously continued in West Texas into November. Calvert concluded that most of the fall breeding activities occurred west of Austin on *A. latifolia*. Prysby and Oberhauser (2004) found evidence of a strong fall fifth generation of monarchs across Texas from late September to October on *A. curassavica* grown in gardens. Batalden and Oberhauser (2015) found a peak in monarch eggs around the second week of September at 30°N, which includes the South-Central Texas region, and they attributed this oviposition to pre-migrants.

The main goal of this study was to analyze the spatio-temporal distribution of adult pre-migrants and relate it to the fifth generation in the South-Central US. The objectives are to 1) separate early adults from the Journey North fall adult sighting data from 1997-2017 in both the Central and Eastern flyways, 2) create kernel density estimate models (KDEMs) for pre-migrants and fall immature monarchs separately for each flyway, and, finally, 3) link early migration with the fifth generation in the South-Central US.

Methods

Journey North monarch fall adult sightings (1997-2017) and roost observations (2002-2017) (Fig. VI.1) were used to separate pre-migrants from the main migrant population and to estimate timing of fall migration along the migration pathways. We created a three-dimensional (3D) second-order polynomial regression model to separate pre-migrants from the main mass of fall migrating monarchs for each flyway (Fig. VI.2). We used the boundary between the Central and Eastern flyways as defined in Tracy et al. (2019) (Section I) to divide roost and adult records into two subsets. Based on the literature, timing of migration is different between the flyways. The movement pattern along the flyways is not only latitudinal, but also longitudinal, especially on the Eastern flyway. The fitted 3D model to the fall adult sighting data represents the average timing of fall migration at different latitudes and longitudes (Fig. VI.2.). We calculated standard deviation (sd) for latitude and longitude by deciles for roosting data. Twice the average \pm sd covers ~95% of the variation in the roosting data. Accordingly, we assign a threshold for pre-migrants as the fitted function minus two times the sd from the roost data decile values.

The migrants and pre-migrants were separated using the threshold day of the year for each geocoordinate given the model fitted functions. For year to year consistency, seven-day week periods since 1 January were used to group the data for mapping. The fall sighting data was limited to the beginning of August each year, corresponding with week 30. We created weekly KDEMs (Tracy et al. 2019; Section I) for the pre-migrants and the main migration in order to approximate the location of the movement each week of the fall. The resulting projections were estimates of the average spatio-temporal patterns of migration and pre-migration over the years, which varies between years.

Results

Separate weekly KDEMs show the location of pre-migrants and migrants in the Central and Eastern flyways Figs. VI.3-4). Pre-migrants are present from the beginning of August in both flyways and their presence is gradually reduced southwards over time as they are replaced by main migrants from the north. Using the 3D spatio-temporal models for any given day, we could project the identity of surveyed monarchs as pre-migrants or migrants (Fig. IV.5).

Discussion

We found a relatively high number of pre-migrants are present in Texas in August, with the numbers increasing during the following weeks. The results are in line with information on average timing of fall migration in Texas. Some adults classified as pre-migrants along the coastal areas and in Mexico may represent year-round resident breeders. The modeled pre-migrants arriving in the South-Central US before the main migration can contribute to the fifth generation. Overlapping of the KDEMs of pre-migrants and main migrants is due to the buffering effect of the seven-day classification of each migrant group. The KDEMs extending into northern latitudes above ca. 37 degrees may include summer breeding monarchs, but an arbitrary geographical border to limit the forming of pre-migrant KDEMs was avoided. These results further confirm previous assumptions on later timing of fall migration of the coastal migrants compared to those along the Central Flyway. Main migrant appeared from week

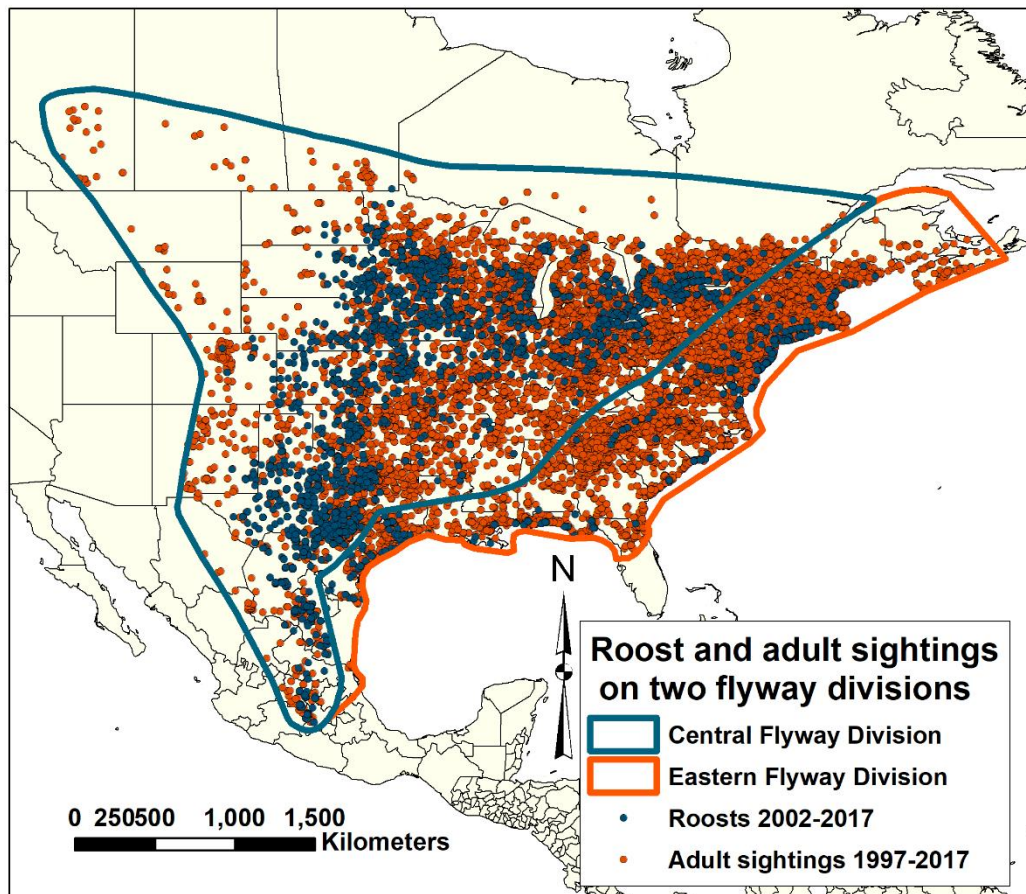


Figure VI.1. Monarch roosts (2002-2017) and adult sightings (1997-2017) from August to November (Journey North 2017).

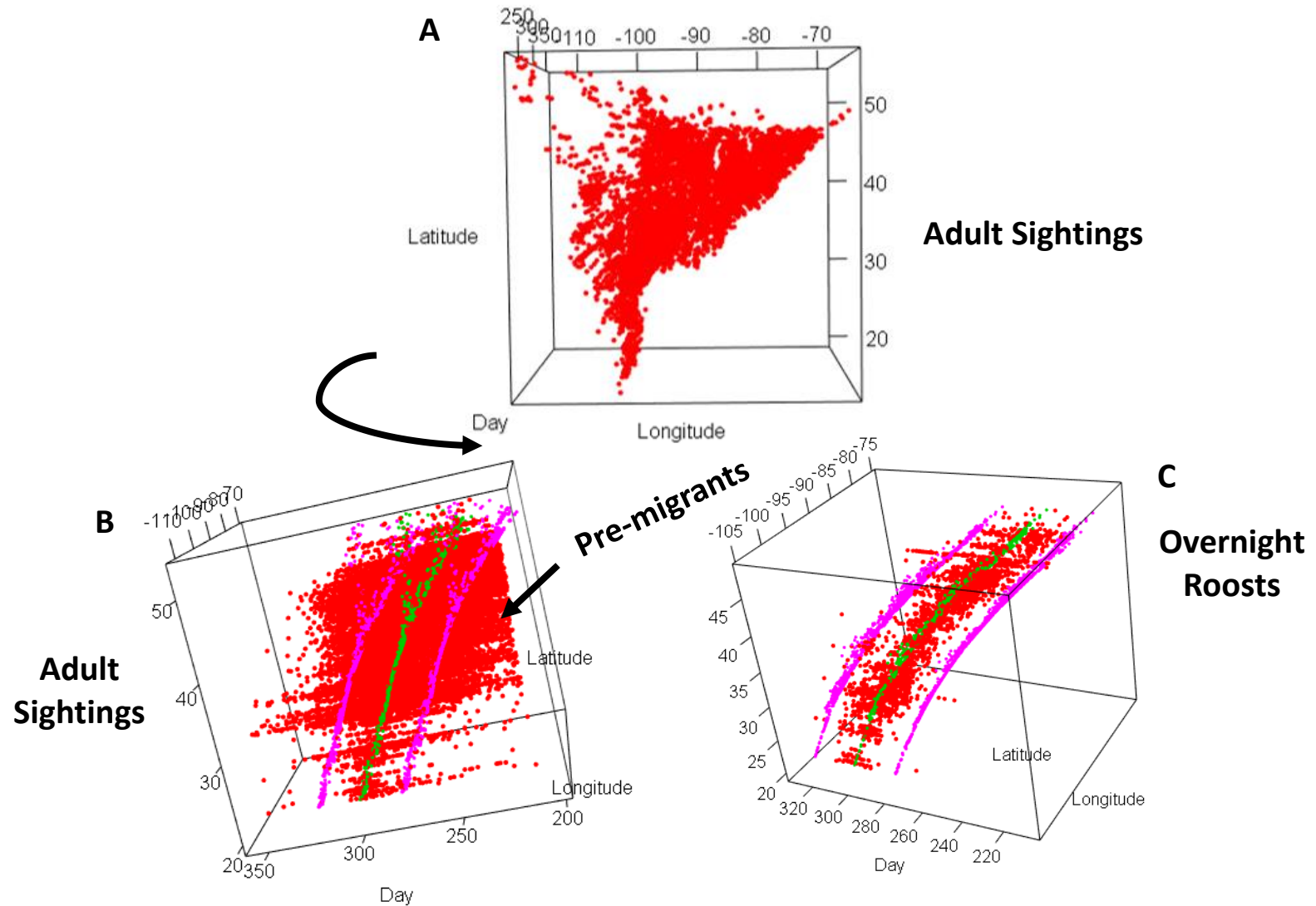


Figure VI.2. (A-B) Spatio-temporal locations (latitude \times longitude \times day of year) of monarch August-December adult sightings (A-B), and (C) overnight roosts within the Central Flyway, including a three-dimensional second order polynomial regression prediction for the spatio-temporal occurrence of roosts (green line) and twice the standard deviation (pink lines) from the regression prediction used to separate pre-migrants from migrants (roosts) by location and day of year (B).

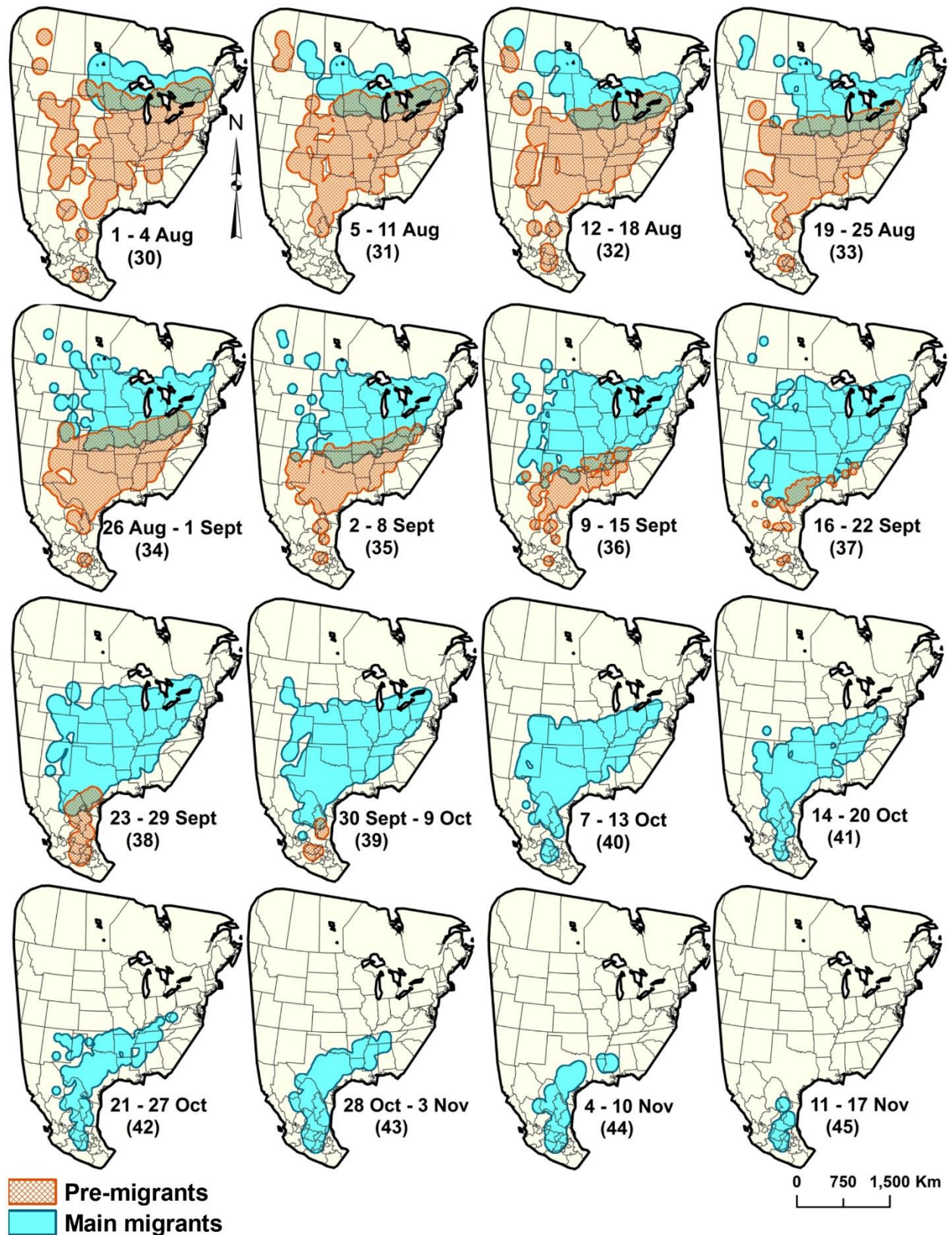


Fig. VI.3. Monarch kernel density estimation models (KDEMs) for pre-migrants and main fall migrants from 2002–2017 combined data (Fig. IV.1) for day-of-year weeks 34 to 45 in the Central Flyway. Models represent minimum frequency consensus training set ensemble of three binary calibrated models developed from a three-fold training data partition.

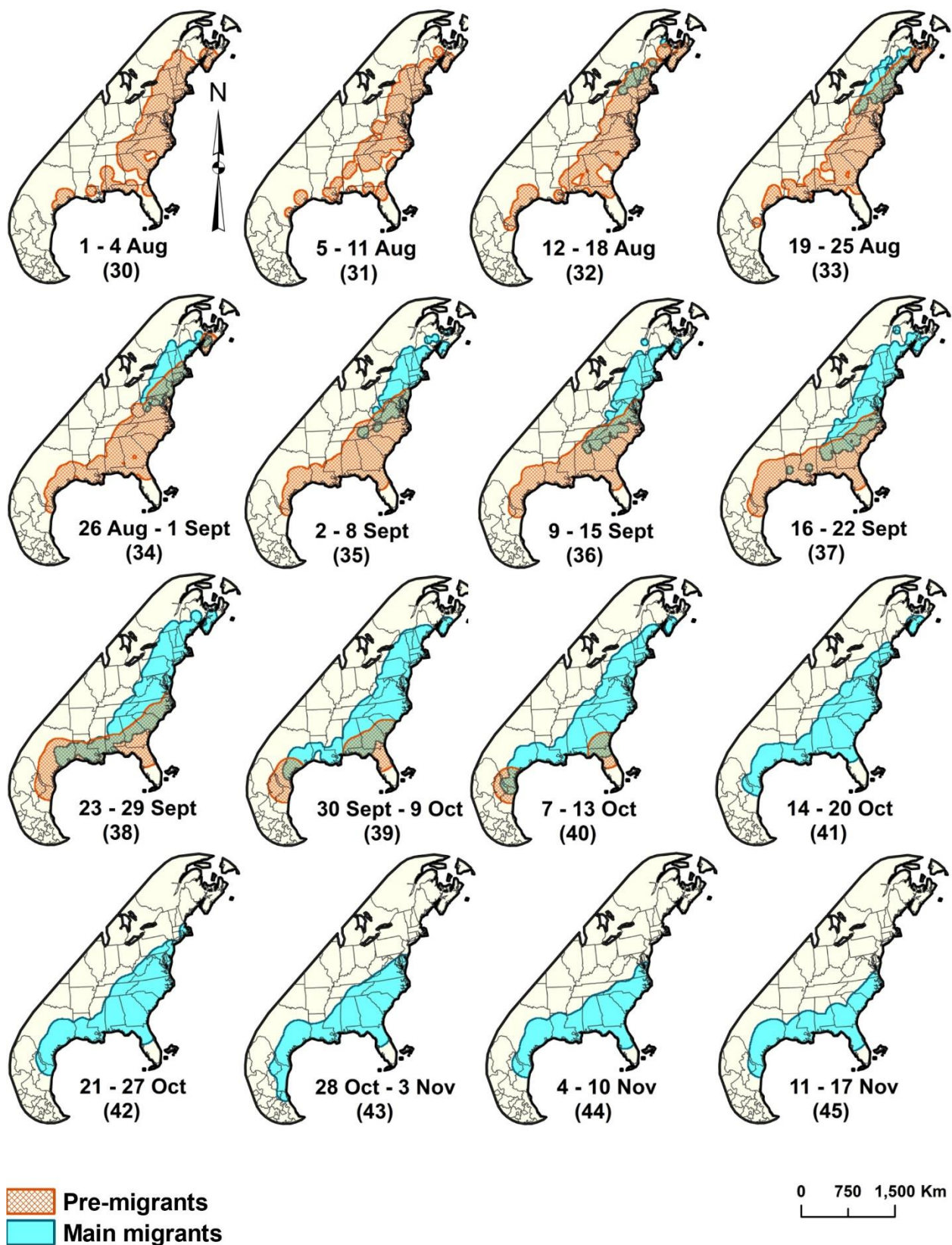


Fig. VI.4. Monarch kernel density estimation models (KDEMs) for pre-migrants and main fall migrants from 2002–2017 combined data (Fig. IV.1) for day-of-year weeks 34 to 45 in the Eastern Flyway. Models represent minimum frequency consensus training set ensemble of three binary calibrated models developed from a three-fold training data partition.

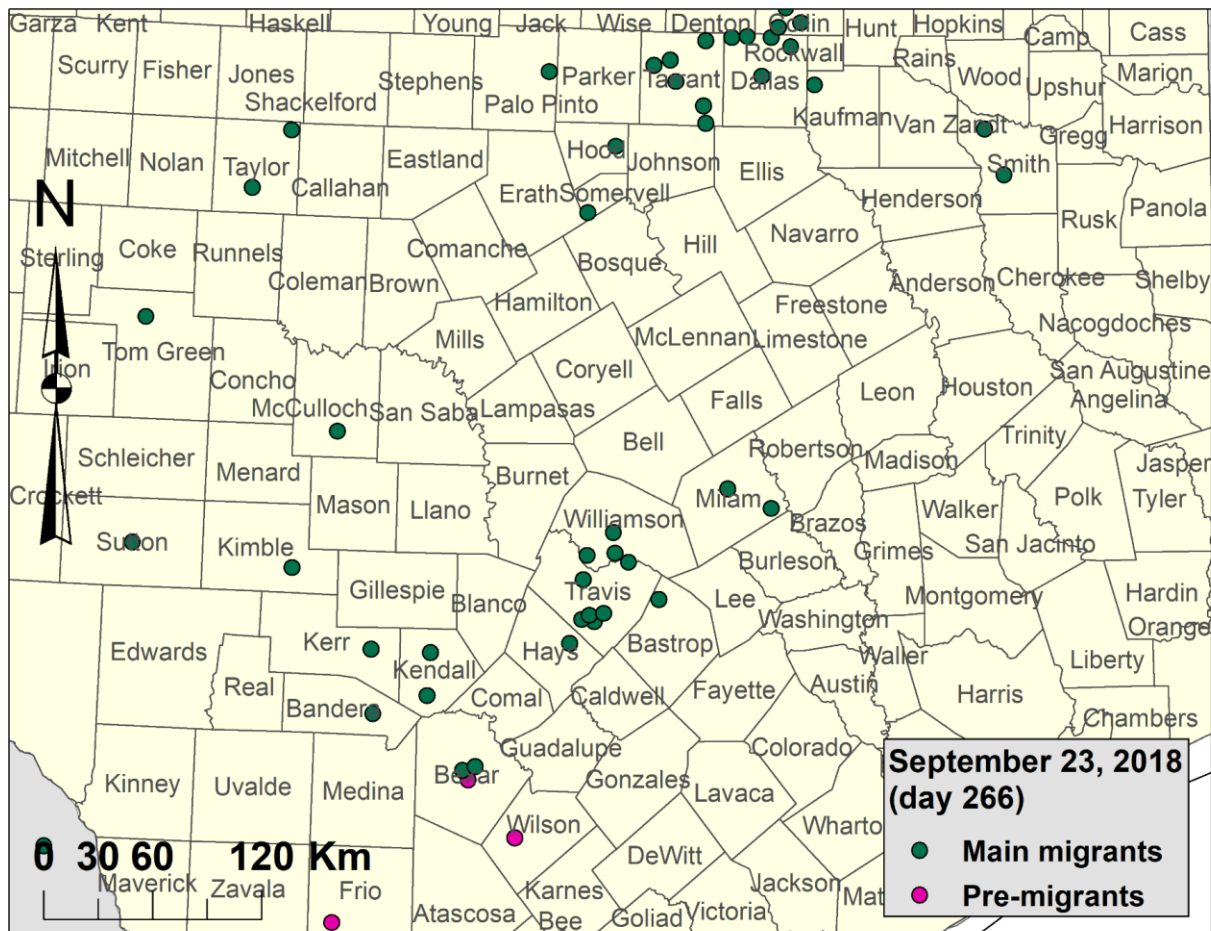


Fig. VI.5. Spatio-temporal projection of adult monarch pre-migrants and main migrants in Central Texas for day of year 266 (23 September for 2018) from 3D model of the Central Flyway.

30 in the Central Flyway week 33 in the Eastern Flyway. In the future, these KDEM maps can be incorporated into risk assessment of fall migration and the fifth generation in Texas. The results on pre-migrants can be linked to the appearance of the fifth generation in Texas and South-Central US.

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SECTION VII: CONTRIBUTION OF FIFTH GENERATION MONARCH BUTTERFLIES TO OVERWINTERING POPULATION

Introduction

Winter-breeding activity has been recorded along the Gulf Coast of Texas, primarily on tropical milkweed (*Asclepias curassavica*), which is a non-native milkweed species that is available commercially. There is concern about the increased risk of *Ophryocystis elektroscirrha* (OE) infection for these individuals, and potential interactions with spring migrants as they return to Texas (Satterfield et al. 2015). The extent of winter-breeding activity is not clear, including whether native milkweed species support monarch eggs and caterpillars during the winter.

Methods

Surveys were conducted during the winters of 2016-17 and 2017-18 (December and January both winters), as well as December 2018, to evaluate habitat use by winter-breeding monarchs. The number of milkweed plants on 5m x 50m transects (field and roadside sites) or within each site (gardens) were counted and the number of eggs and instars were recorded. We also tagged adult monarchs at each site, but have few tag recoveries to date (but additional tag recoveries could be reported in subsequent years to Monarch Watch).

Results/Discussion

Asclepias curassavica was the dominant milkweed species present in gardens, although a few *A. oenotheroides* plants were present at a few sites. Field and roadside sites contained primarily native milkweed species (*A. viridis*, *A. oenotheroides*, *A. perennis*), with the exception of a few *A. curassavica* present at 2 fields sites during the winter of 2017-18. Milkweed was mainly present at garden sites during the 2016-17 winter, but was present at more field and roadside sites during the winter of 2017-18 (Fig. VII.1), likely re-emergence in response to heavy rainfall from Hurricane Harvey earlier that year. Monarchs primarily used garden sites for egg laying (Fig. VII.2). No eggs were found at roadside sites, although one 5th instar was observed on *A. perennis* in December 2017 (Fig. VII.3). Few eggs were found at field sites (compared to gardens) in December, and no eggs were observed at field sites in January. Eggs at field sites primarily occurred on *A. oenotheroides*, although one egg was observed on *A. viridis* in December 2017. These results suggest that most winter-breeding activity occurs in garden sites on *A. curassavica*, but that native milkweeds may be present late in the year under some conditions (e.g., the 2017-18 winter following Hurricane Harvey). Additional research is needed to evaluate the role of native and non-native milkweeds in winter-breeding activity, especially how out-of-season native milkweeds could potentially influence the observed patterns and subsequent milkweed availability in the spring (e.g., do native milkweeds that re-emerge late in the year following heavy rainfall events emerge again the following spring?).

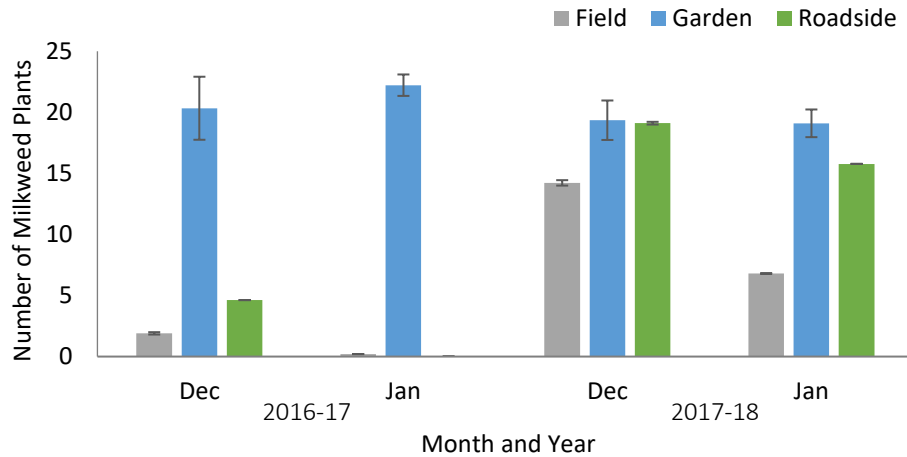


Figure VIII.1. Mean number (\pm SE) of milkweed plants on each 5m x 50m transect (field and roadside sites) or within each site (gardens) during winter surveys. Garden sites primarily contained *A. curassavica*, whereas field and roadside sites primarily contained native milkweed species (*A. viridis*, *A. oenotheroides*, *A. perennis*). Note: The number of milkweed plants per transect in field and roadside sites is not directly comparable to the number of milkweed plants in garden sites, since all plants in a discrete area were counted at garden sites.

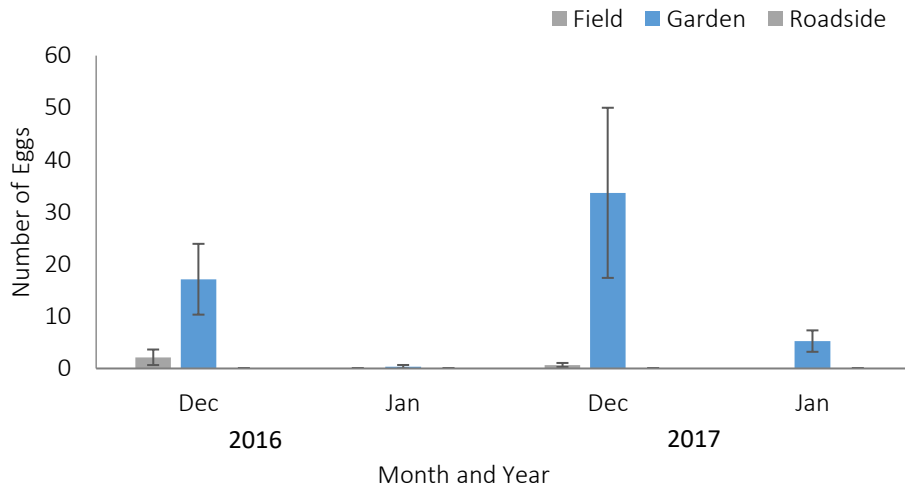


Figure VIII.2. Mean number (\pm SE) of monarch eggs on milkweed plants within each 5m x 50m transect (field and roadside sites) or within each site (gardens) during winter surveys.

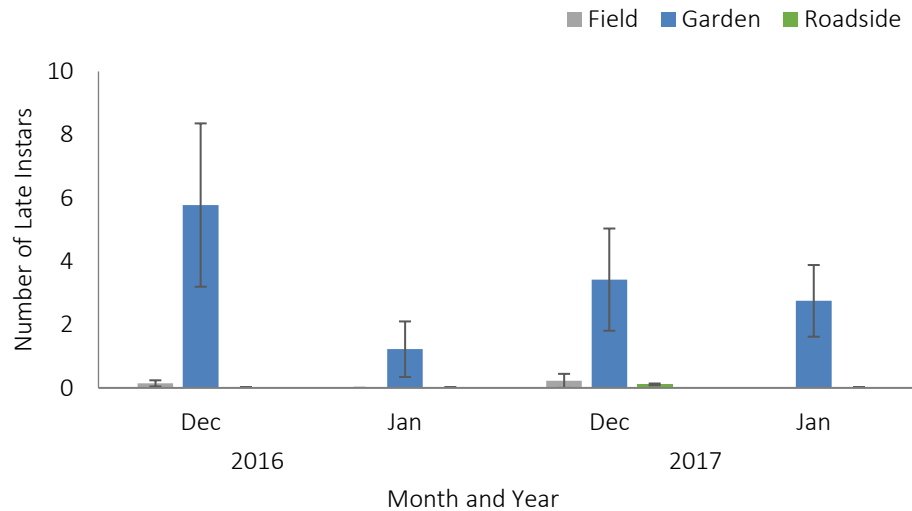


Figure VIII.3. Mean number (\pm SE) of monarch late instars on milkweed plants within each 5m x 50m transect (field and roadside sites) or within each site (gardens) during winter surveys.

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SECTION VIII: HABITAT USE BY PRE-MIGRANT MONARCHS AND THEIR FIFTH GENERATION OFFSPRING

Introduction

The immature monarchs present in Texas during September and October are considered part of the 5th generation that is produced by pre-migrant monarchs (late or fall breeding monarchs that move south early, prior to the fall migration, and reproduce in Texas and surrounding states; Calvert 1999, Baum and Sharber 2012, Mueller and Baum 2014). It is currently not known what proportion of the overall population is comprised of pre-migrants, or how important this 5th generation is to the overall population. However, recent research based on stable isotopes suggests that monarchs produced in the south-central US contribute, on average, 11% to the overwintering population each year (Flockhart et al. 2017).

Methods

To evaluate the timing of 5th generation activity, habitat use by pre-migrant monarchs (i.e., presence of 5th generation monarch eggs and instars), and milkweed availability for 5th generation monarchs, surveys were conducted from late August/early September through early November of 2016, 2017, and 2018. Surveys were in fields, gardens, and roadsides at sites identified to contain milkweed (Figure VIII.1). The number of milkweed plants on 5m x 50m transects (field and roadside sites) or within each site (gardens) were counted and the number of eggs and instars were recorded. Data are presented for 2017 and 2018, during which surveys were conducted approximately once every two weeks (surveys were less frequent in 2016).

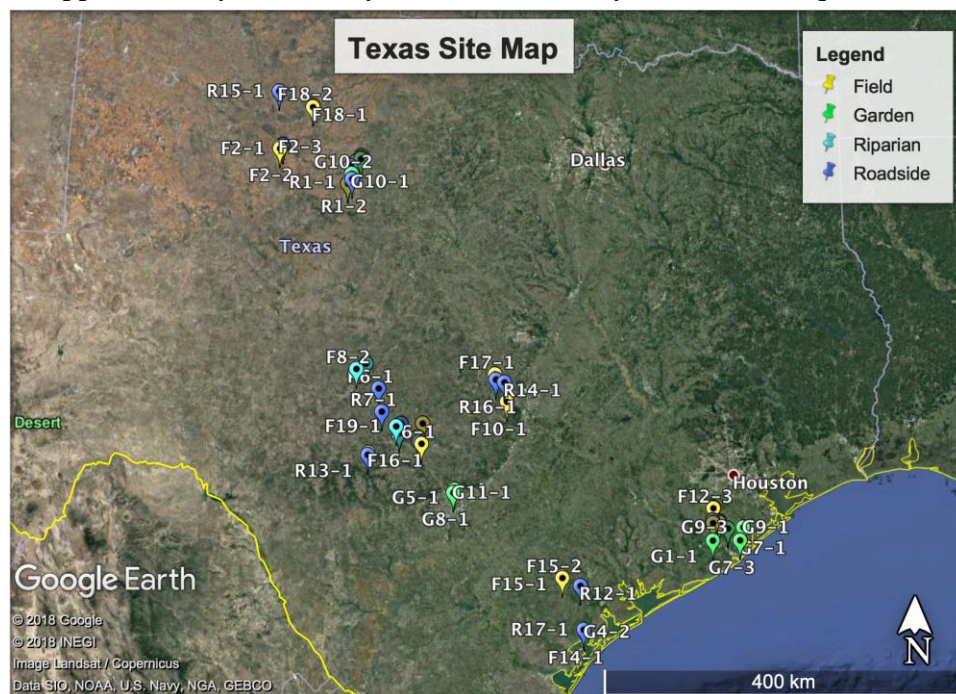


Figure VIII.1. Location of sites within the three study regions (Abilene, Kerrville, and Gulf Coast). Designations for site types include field (F), garden (G), and roadside (R).

Results/Discussion

The dominant milkweed species varied by region, with *A. latifolia* being most abundant in the Abilene region, *A. asperula* in the Kerrville region, and *A. viridis* in the Gulf Coast region. Other milkweed species observed included *A. oenotheroides* in all three regions, and *A. perennis* in fields and roadsides in the Gulf Coast region. Kerrville was the only region that included riparian sites, which contained *A. incarnata* plants. The number of milkweed plants per transect in field and roadside sites is not directly comparable to the number of milkweed plants in garden and riparian sites, since all plants in a discrete area were counted at garden and riparian sites. In general, roadside sites tended to contain more milkweed plants per transect than field sites, with the exception of sites in the Kerrville region in 2018 (Fig. VIII.2). Milkweed availability tended to decline across sampling periods (Figs. VIII.2-3). Eggs were most abundant in garden sites in 2018, and patterns in egg laying activity varied by region and year (Fig. VIII.4). Late instars were also most abundant in garden sites, and also varied in distribution across regions and years (Fig. VIII.5). Monarchs used all of the common milkweed species observed (*A. asperula*, *A. incarnata*, *A. latifolia*, *A. oenotheroides*, *A. perennis*, and *A. viridis*). Based on this summary of the data, garden sites seem to be the main sites used by monarchs (Figs. VIII.5-6), which reflects that garden sites are more likely to contain eggs and caterpillars than other types of sites (i.e., fewer sites with no monarchs), and may contain high numbers of monarchs. OE infection rates (see Section IX) were also higher in garden sites. **Monarchs also use other types of sites, and we are working on additional ways to summarize the data that may provide a better overview of the data in the context of supporting monarch conservation needs.** Additional research is needed to evaluate the spatial and temporal distribution of 5th generation monarchs, including identifying factors that influence the size and origin of the pre-migrant population each year.

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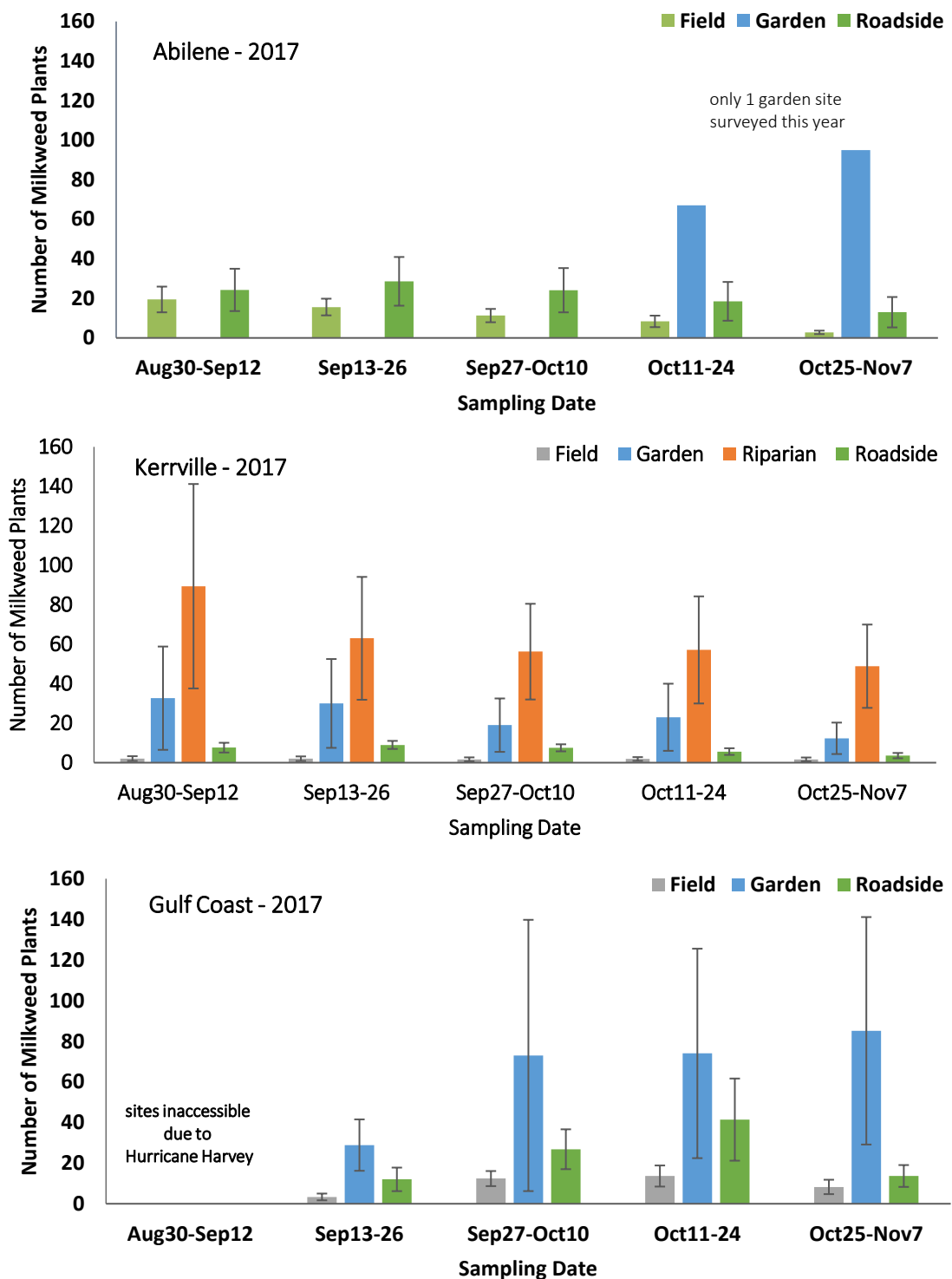


Figure VIII.2. Fall 2017 mean number (\pm SE) of milkweed plants on each 5m x 50m transect (field and roadside sites) or within each site (gardens) by region (Fig. VIII.1).

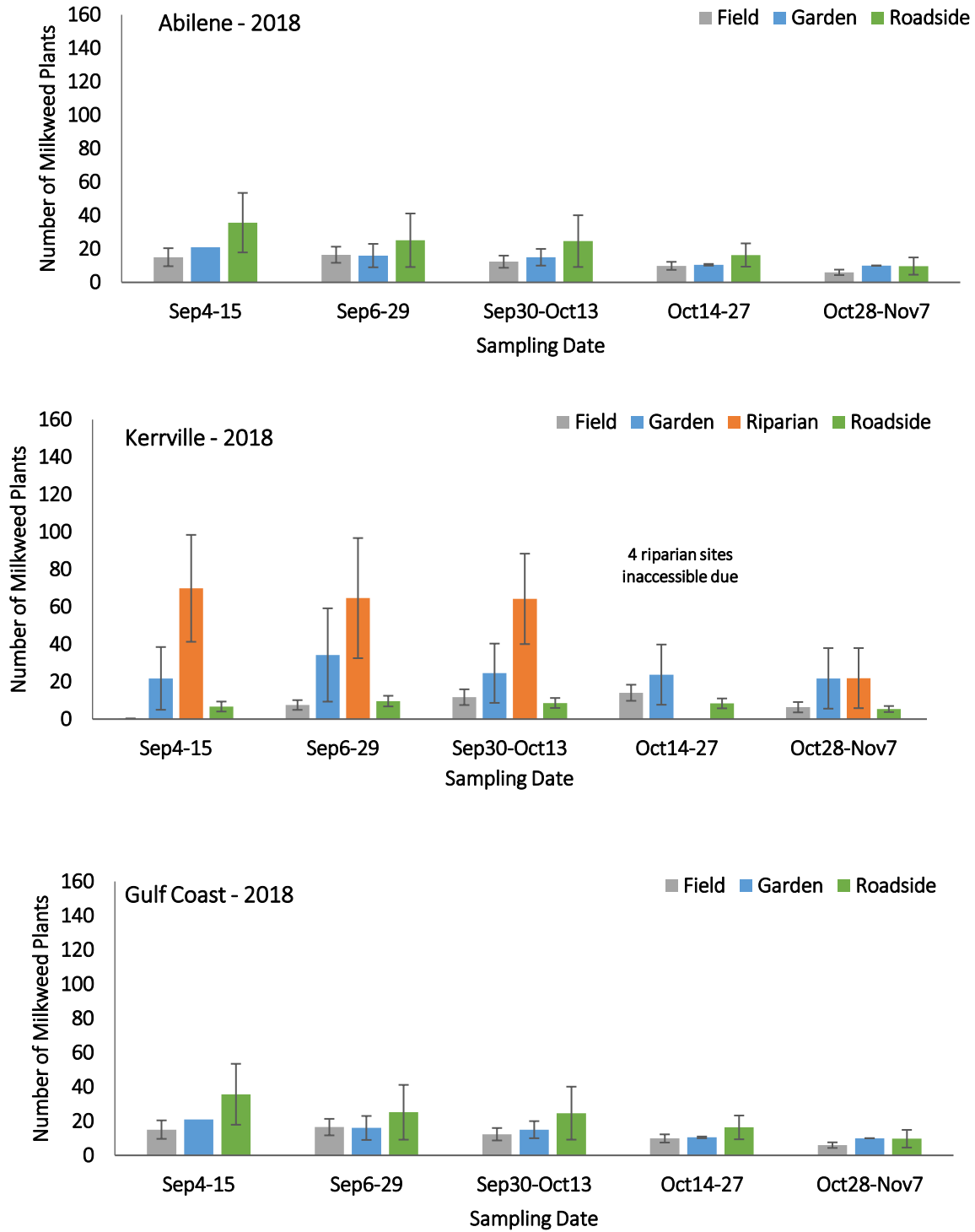


Figure VIII.3. Fall 2018 mean number (\pm SE) of milkweed plants on each 5m x 50m transect (field and roadside sites) or within each site (gardens) by region (Fig. VIII.1).

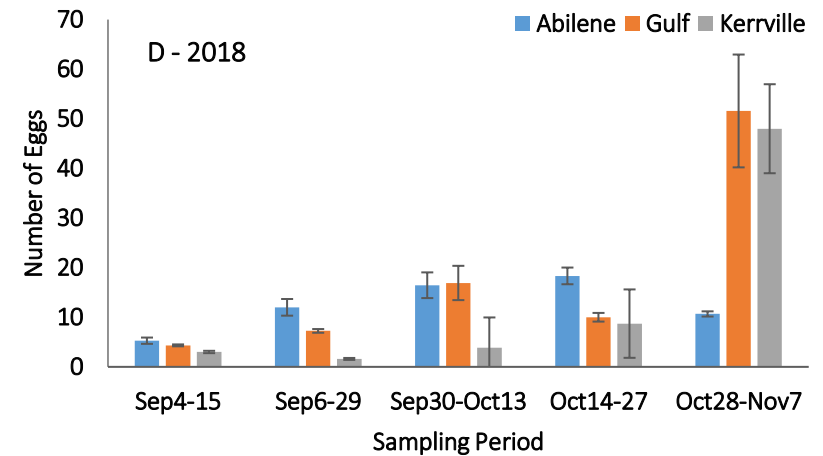
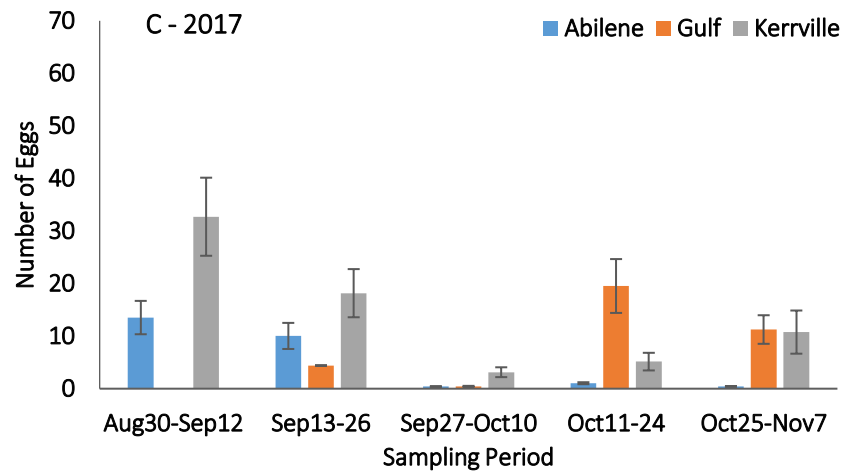
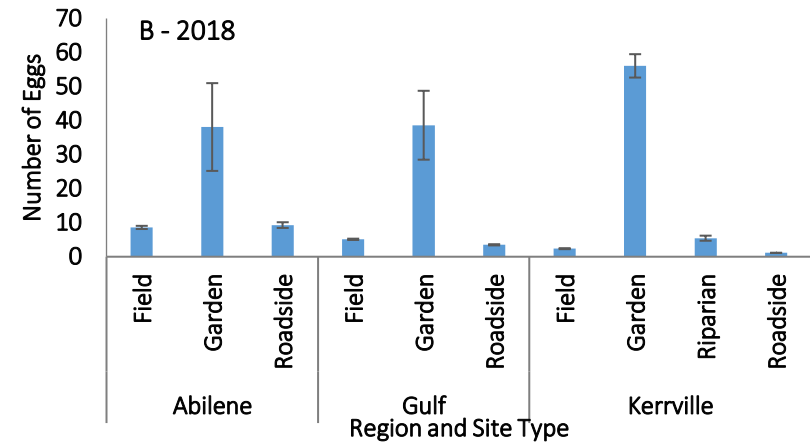
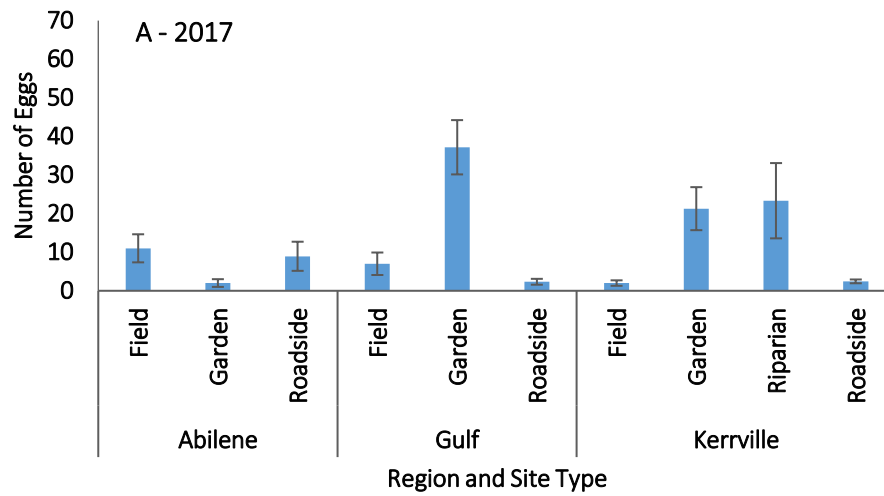


Figure VIII.4. Mean number (\pm SE) of monarch eggs on milkweed plants within each 5m x 50m transect (field and roadside sites) or within each site (gardens) during fall surveys by region and site type for 2017 (A) and 2018 (B) and by sampling period for 2017 (C) and 2018 (D).

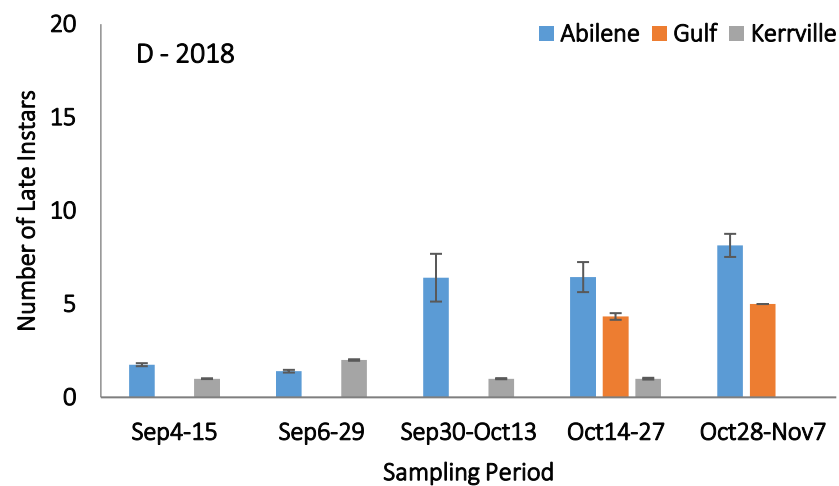
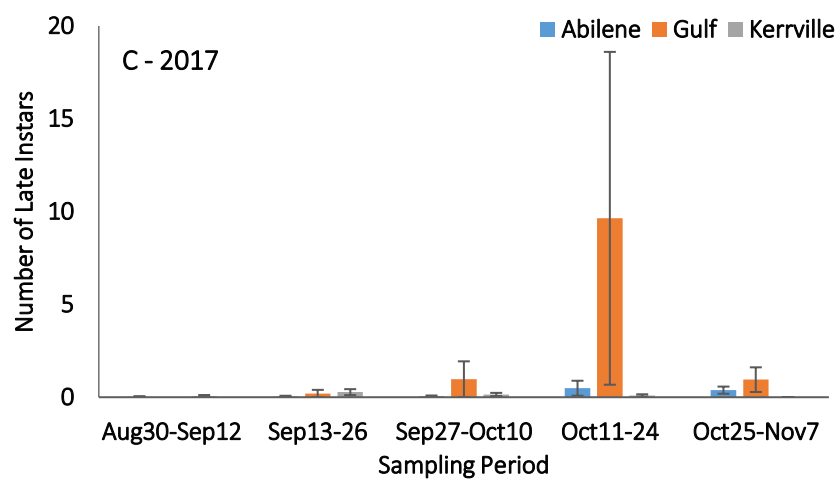
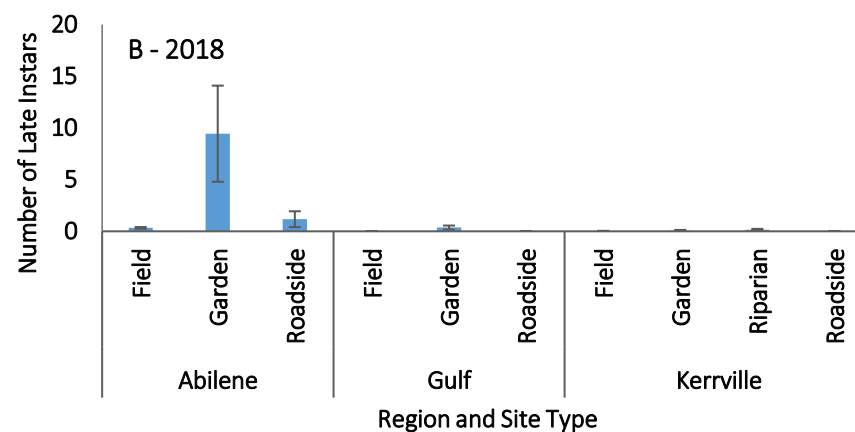
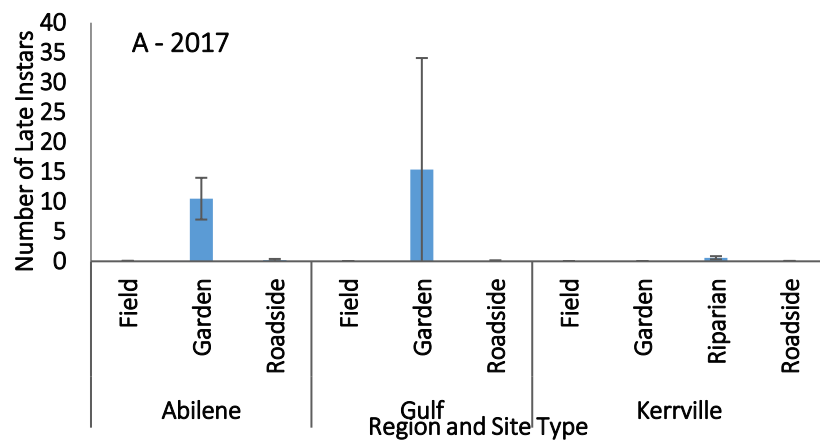


Figure VIII.5. Mean number (\pm SE) of monarch late instars on milkweed plants within each 5m x 50m transect (field and roadside sites) or within each site (gardens) during fall surveys by region and site type for 2017 (A) and 2018 (B) and by sampling period for 2017 (C) and 2018 (D).

SECTION IX: IMPACT OF OPHRYOCYSTIS ELECTROSCIRRHA (OE)

Introduction

Ophryocystis elektroscirrha (OE) is an obligate spore-forming protist that infects monarchs. When infected, adult monarch butterflies are externally covered with thousands of dormant spores, and tend to not live as long or fly as well. OE infection rates increase across the breeding season and then decline during the migration, as infected individuals are not expected to survive the migration and/or winter (Altizer et al. 2000, Bartel et al. 2011). Data collected by citizen scientists for Project Monarch Health (presented in Thogmartin et al. 2017) suggest that the final generation of monarchs that migrates to Mexico has an infection rate of approximately 8.7%. Tachinid flies are another parasite of monarchs. They are considered generalist parasitoids of monarch caterpillars and other hosts, and kill the host when they emerge from late instar caterpillars. Previous research based on citizen scientists participating in the Monarch Larva Monitoring Project has found that approximately 16% of late instars are infected across the monarch's range (Oberhauser et al. 2017). Fly parasitism is variable among years, regions, and even nearby sites, and seems to track the monarch population (a high year for monarch abundance is often followed by a high year of fly parasitism).

Methods

To evaluate the impact of OE on 5th generation monarchs and the offspring of winter-breeding adults, late instars collected from surveys for studies in Sections VI and VIII were reared to adults and sampled for OE using a standard protocol. We also report data on tachinid fly parasitism as a source of mortality for 5th generation monarchs.

Results/Discussion

For 5th generation monarchs (late instars collected from late August through early November), OE infection rates ranged from a low of 9% in 2016 to a high of 14% in 2017 (Fig. IX.1). OE infection rates were highest in garden sites each year. However, OE infection rates were 1.5 (2017) to 2.4 (2016) times higher when calculated based on the number of late instars that emerged as adults instead of the total number of late instars collected (Fig. IX.2). Tachinid fly parasitism ranged from a low of 22% in 2017 to a high of 48% in 2016 (Fig. IX.1), and varied across site types. For the offspring of winter-breeding monarchs (late instars collected during December and January), tachinid fly parasitism rates were very high in December (more than 2/3 of late instars were parasitized) and lower in January (Fig. IX.3). OE infection rates increased from December to January each year (3.75 times higher in January in 2017 and 2.3 times higher in January in 2018; Figure 10). However, OE infection rates were more than 5 times higher in December and 2 to 3 times higher in January when calculated based on the number of late instars that emerged as adults instead of the total number of late instars collected (Fig. IX.3). However, hard freezes occurred in January of both years, and it is expected that few, if any, monarchs survived. Additional research is needed to evaluate survival of winter-breeding monarchs and their offspring across years, and to assess the extent of interactions with spring migrants.

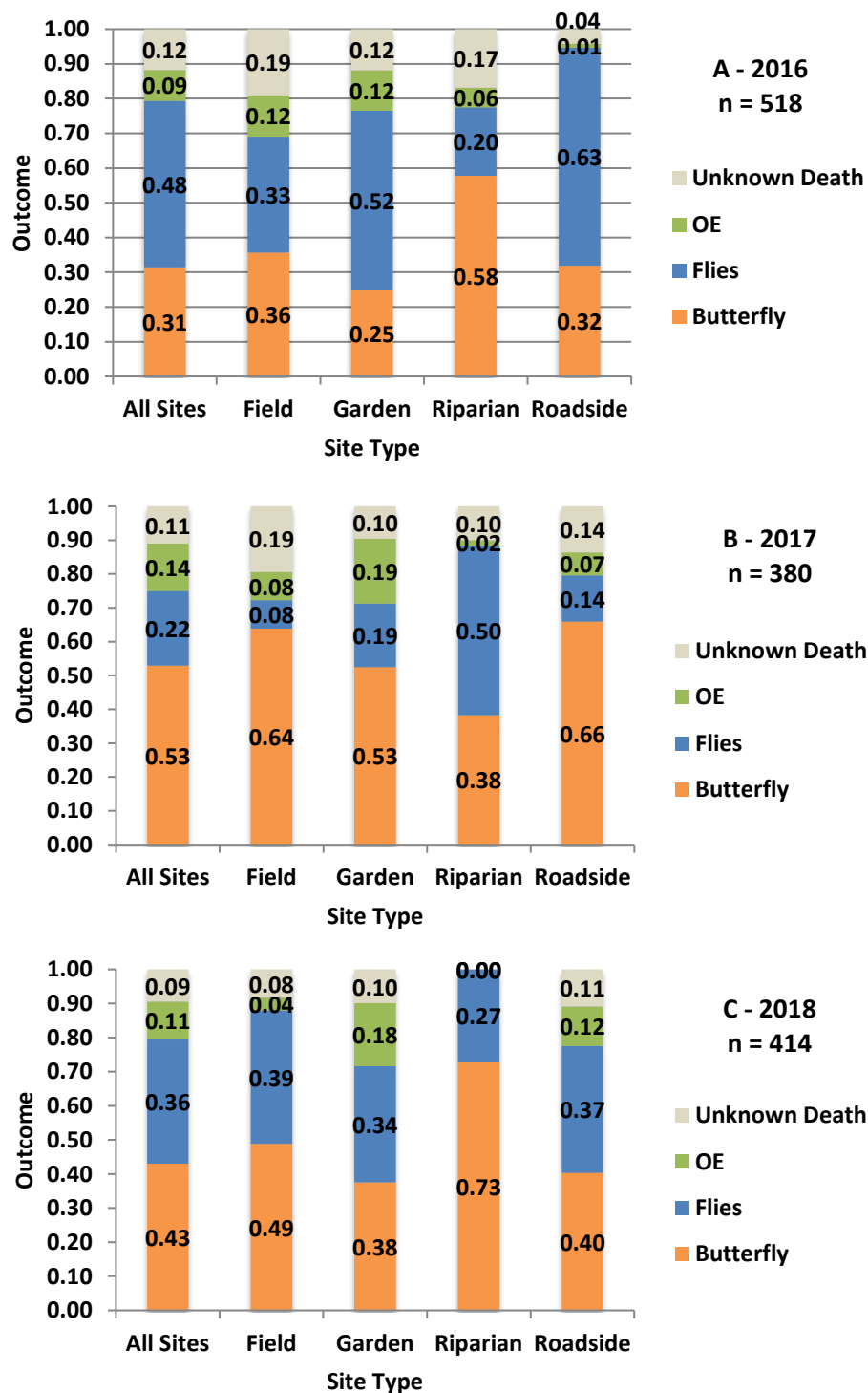


Figure IX.1. Outcome (proportion of individuals in each category) for reared late instars for 2016 (A), 2017 (B), and 2018 (C) overall (all sites combined) and by site type. Butterfly refers to those that eclosed and were not infected with *Ophryocystis elektroscirrha* (OE), flies refers to those that died in the larval or pupal stage from tachinid flies, OE refers to those with OE infection, and unknown death refers to those that died of unknown causes. Overall samples sizes (n) are provided for each year.

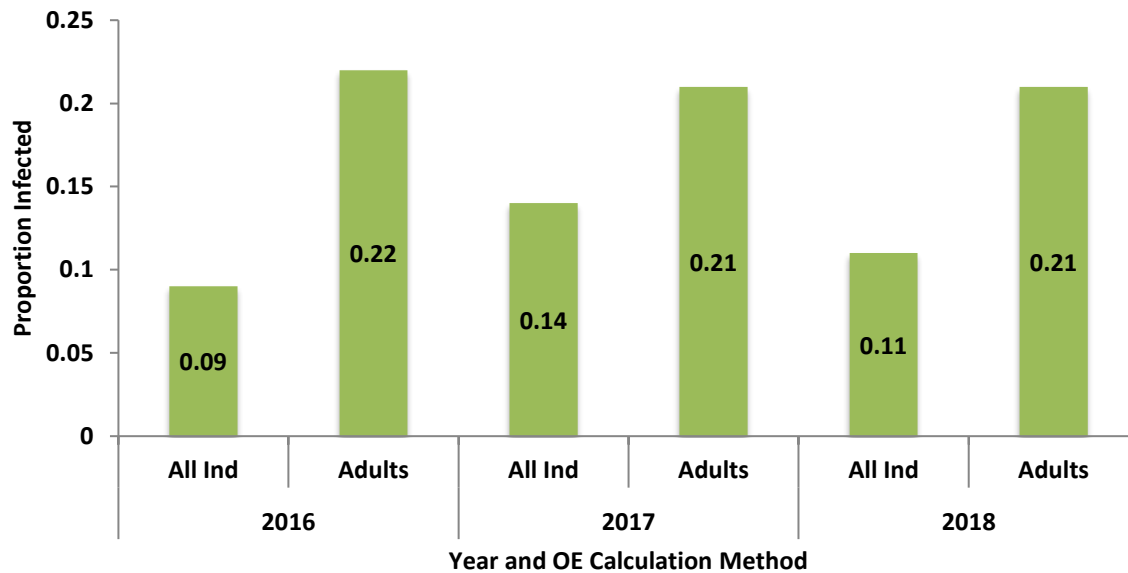


Figure IX.2. *Ophryocystis elektroscirrha* (OE) infection rates when calculated with all collected late instars (“All Ind”) included (same as in Fig. IX.1) and with only late instars that emerged as adult butterflies (“Adults”) included.

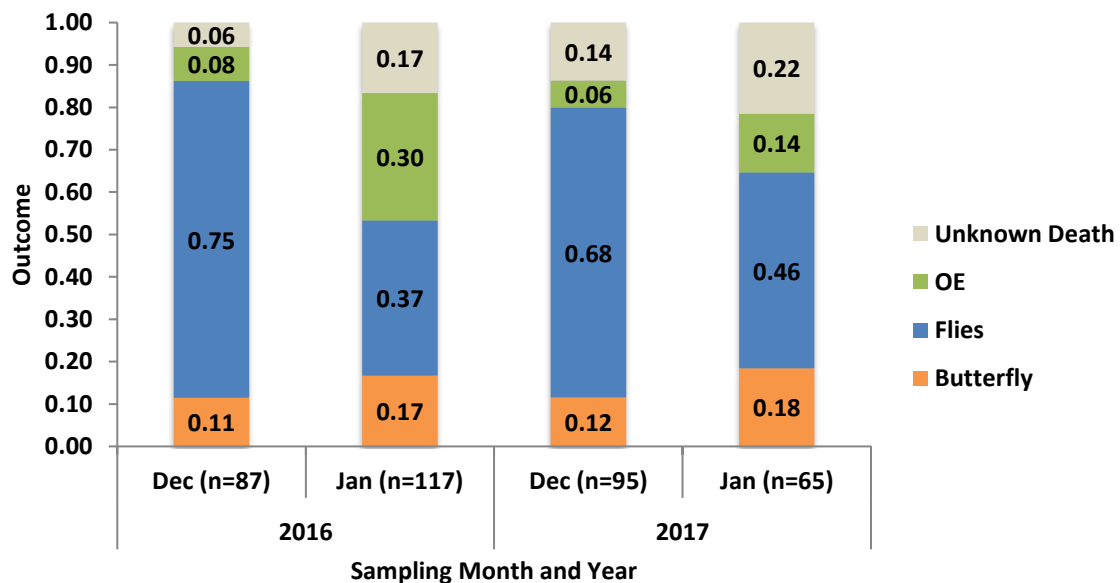


Figure IX.3. Outcome (proportion of individuals in each category) for reared late instars during winter sampling periods for 2016-17 and 2017-18. Overall samples sizes (n) are provided for each month. If *Ophryocystis elektroscirrha* (OE) infection rates were calculated for only those individuals that emerged as adult butterflies, the rates would be 41% (Dec 2016), 64% (Jan 2017), 35% (Dec 2017), and 43% (Jan 2018).

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ADDITIONAL RESEARCH NEEDS

The project has been a blend of empirical research and spatial modeling. Further synthesis and interpretation of the results of the various studies in this report can provide a broader landscape ecology/land-use management perspective for our findings. The goal and objective of the project were tailored specifically to address the criteria used in the Species Status Assessment (SSA) process by the US Fish & Wildlife Service (F&WS). The fundamental omission for the project is a tailored synthesis and interpretation of the results in the context of the SSA. This synthesis was beyond the scope of the existing project, but would provide significant and new information relevant to a status of the monarch butterfly. None of the results from the project have yet been considered in the draft SSA document.

Several specific future research needs can be identified from the various sections of this report. The spatial analysis of hazards during the monarch fall migration revealed the mosquito spraying, especially along the Eastern flyway and Coastal Funnel, is probably detrimental to monarchs and requires further investigation, both for impacts to monarchs and potential offsetting mitigation measures (Section I). Our fall monarch roadkill study revealed significant mortality in the Central Funnel of Texas, and further studies are needed that include the Coastal Funnel and larger areas of the Central Funnel (Section II). Our landscape risk analyses indicate significant areas are subject to habitat loss to Cultivated Crop land cover for both important milkweed host plants and nectar plants in the South-Central US (Sections III and V). Further study is needed to document potential benefits to milkweeds and nectar plants from ongoing and expanded habitat conservation and restoration measures. Knowledge of the seasonal and regional distribution of monarch larvae on various milkweed hosts across the South-Central US would greatly benefit from broader field surveys, especially in the far western and eastern parts of the state, and additional analyses of current data (Section IV). The newly developed monarch migrant and pre-migrant spatio-temporal models can be further utilized for guiding future risk analyses and conservation planning (Section VI). Additional research is needed to evaluate the role of native and non-native milkweeds in winter-breeding activity, especially how out-of-season native milkweeds could potentially influence the observed patterns and subsequent milkweed availability in the spring (e.g., do native milkweeds that re-emerge late in the year following heavy rainfall events emerge again the following spring?) (Section VII). Additional research is also needed to evaluate the spatial and temporal distribution of 5th generation monarchs during the fall, including identifying factors that influence the size and origin of the pre-migrant population each year (Section VIII). Further study is needed to evaluate survival of winter-breeding monarchs and their offspring across years in relation to *Ophryocystis elektroscirrha*, and to assess the extent of interactions with spring migrants (Section IX).

LITERATURE REVIEW:
MONARCH BUTTERFLY (*DANAUS PLEXIPPUS*) POPULATION STATUS,
TRENDS, AND THREATS IN TEXAS

Tuula Kantola, James L. Tracy, Kristen A. Baum, and Robert N. Coulson

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1. Introduction

Population decline of the monarch butterfly (*Danaus plexippus plexippus*) on its overwintering grounds in central Mexico during the last two decades has led to it being considered for listing as threatened under the Endangered Species Act (Center for Biological Diversity et al. 2014; also see Thogmartin et al. 2017 for estimates of monarch population size on the overwintering grounds). Furthermore, the species long distance migration has been considered an endangered phenomenon (Brower et al. 2012). The monarch butterfly migrates from overwintering grounds in central Mexico to summer breeding grounds in the Upper Midwest and southern Canada. Monarchs migrate through Texas during both spring and fall migration, and reproduce in this region during spring and fall breeding periods. Therefore, Texas has been identified as critical for conservation efforts, both in terms of providing milkweed for reproduction and nectar sources to fuel the spring and fall migration.

Spring migrants first reach Texas in mid-March and lay the first generation of eggs as they continue to move North. Because of this critical location between the overwintering grounds and the summer breeding grounds, Texas plays a critical role in spring migration. Immature monarchs are also present in Texas from September and through early November, and are considered to be part of the 5th generation that is produced by pre-migrant monarchs (also referred to as late or fall breeding monarchs that move south early, prior to migration, and reproduce in Texas and surrounding states; Calvert 1999, Baum and Sharber 2012, Mueller and Baum 2014). It is currently not known what proportion of the overall population is comprised of pre-migrants, or how important this 5th generation of monarchs is to the overall monarch population (but see Flockhart et al. 2017). It is possible that the importance of this 5th generation has changed with milkweed loss in the Upper Midwest or that the importance varies among years. Winter-breeding monarchs are also present along the Gulf Coast in some years, and this is thought to be a relatively recent phenomenon, although coastal overwintering (but not breeding) monarchs have been observed for much longer (Satterfield et al. 2015). Winter-breeding monarchs are typically associated with the presence of tropical milkweed (*Asclepias curassavica*) and have higher levels of infection with *Ophryocystis elektroscirrha* (OE) (Satterfield et al. 2015) than coastal overwintering monarchs or those monarchs that overwinter on the overwintering grounds in central Mexico. Tropical milkweed is a non-native species that can remain green year-round in warm areas, whereas as native milkweed species in these same areas typically exhibit an annual cycle that includes a period of senescence during cooler months. There is concern that interactions between spring migrants and winter-breeding monarchs, such as laying eggs on the same plants or mating, could leave to higher levels of OE infection in the overall monarch population.

Partly due to the migrating nature and iconic status of the monarch butterfly, the species has been studied intensively. Despite the acknowledged importance of Texas for the monarch, studies on monarchs or their habitats, or risk factors focusing on this region are relatively few. Research in

identifying and managing risk factors associated with the monarch decline is critical (Inamine et al. 2016, Flockhart et al. 2017). Addressing factors threatening migratory animals requires integrated information on species movement, survival, and reproduction throughout the annual cycle, as well as species responses to the threats (Taylor and Norris 2010, Flockhart et al. 2017). Various factors threatening monarch populations have been identified. Many of these factors are anthropogenic. (e.g., Oberhauser and Peterson 2003, Bradley and Altizer 2005, Brower et al. 2006). There is no current consensus about the reasons for the population decline or the level of impact by different factors. The results of studies related to threats and vulnerability of monarchs are partly controversial.

2. Current Status and Trends

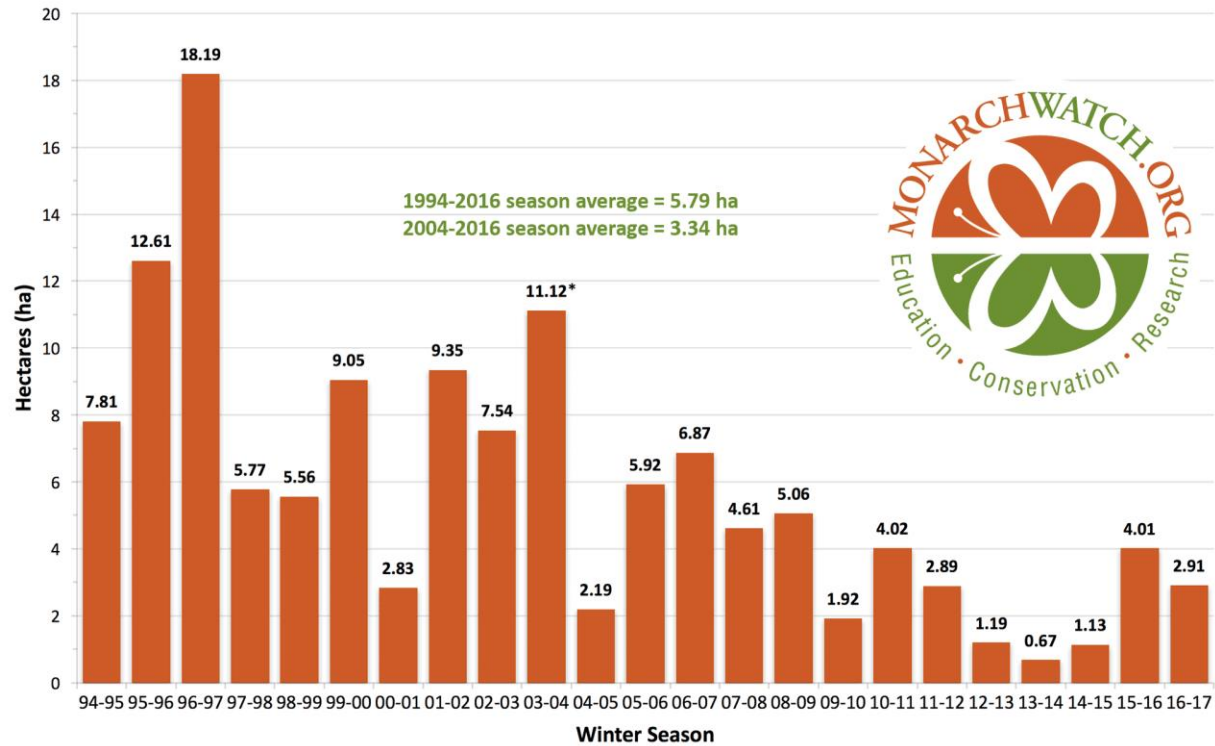
2.1. Population

The estimated hectares of monarch colonies covering the oyamel firs (*Abies religiosa*) at the Mexican overwintering sites serves as a benchmark index of the size of the eastern migrating monarch population since it is not possible to count the number of individual monarchs (Thogmartin et al. 2017). Annual population fluctuations of more than 10-fold have been observed, but a sharp long-term population decline of 80% was recognized from 1993 to 2013 (Vidal and Rendón-Salinas 2014). Since the systematic monitoring of the overwintering monarchs started, the occupied area reached the lowest on record, 0.67 hectares, in the winter of 2013-2014 (Figure 1). In order to estimate the population size, the number of hectares is multiplied by the density of individuals per hectare (Thogmartin et al. 2017). There is considerable uncertainty in evaluating overwintering densities and the estimates have varied from 6.9–60.9 million individuals per hectare (Thogmartin et al. 2017). Thogmartin et al. (2017) suggested a median overwintering density of 21.1 million per hectare. However, they assumed variation in the density within and among years. Furthermore, they suggested that the density of overwintering monarchs is lower on the edges of the sites compared to the central parts. Smaller colonies with higher edge ratio may lead to lower mean densities, and thus, to overestimates of the current population size.

Several population models indicate that the eastern migrating population is at risk of extinction (Flockhart et al 2015, Semmens et al. 2016). Population declines of migrating species such as the monarch can be limited by conditions of their breeding range, non-breeding range, migration success, or as a combination of these key elements (Flockhart et al. 2015). Quantifying these factors is complex and depending on how the migratory connectivity, population processes, and available data are integrated (Flockhart et al. 2015). Population fluctuation at a stage of the annual

Figure 1. Total area occupied by monarchs at the overwintering sites between 1994 and 2017 (Monarch Watch 2017d).

cycle may contribute to the next stage. For example, breeding population fluctuation leads to fluctuation in the number of fall migrants (Prysby and Oberhauser 2004). Variation may also exist in proportion of fall migrating monarchs successfully reaching overwintering sites, affecting the numbers of overwintering monarchs (Garland and Davis 2002). Further, factors, such as predation and weather events at the overwintering sites may reduce the number of spring migrating monarchs. Possible contributors to the fluctuating pattern include abiotic factors, such as temperature, humidity, precipitation, and storm events, natural enemies, host plant quality and availability, and human activities (Prysby and Oberhauser 2004).



2.2. Phenology and Distribution

2.1.1. Overall Pattern

Native distribution of the eastern migratory monarch butterfly ranges from South America to southern Canada, between the Rocky Mountains and the Atlantic Seaboard. The northern distribution is limited by the range of milkweed species (*Asclepias*) (Lynch and Martin 1993). Annual cycle of the monarchs include two long migrations, a breeding season, and an overwintering season (Figure 2). For five months, from November through March, monarchs overwinter in a few oyamel fir sites at high altitudes in Mexico (Brower 1995). At the sites, monarchs aggregate in massive numbers, up to 50 million individuals per hectare (Brower et al. 2004). The overwintering monarchs mate in late winter and early spring. In spring, the migratory generation returns from Mexican overwintering sites (Figure 2). The current consensus is that the majority of monarchs migrate in spring only to the southern USA and lay eggs on the way (Howard and Davis 2004). The next generation completes the northward migration and recolonizes the rest

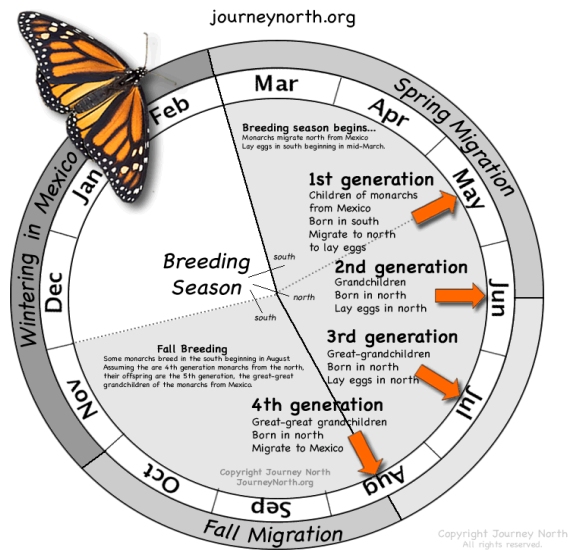


Figure 2. The annual cycle of monarch butterfly

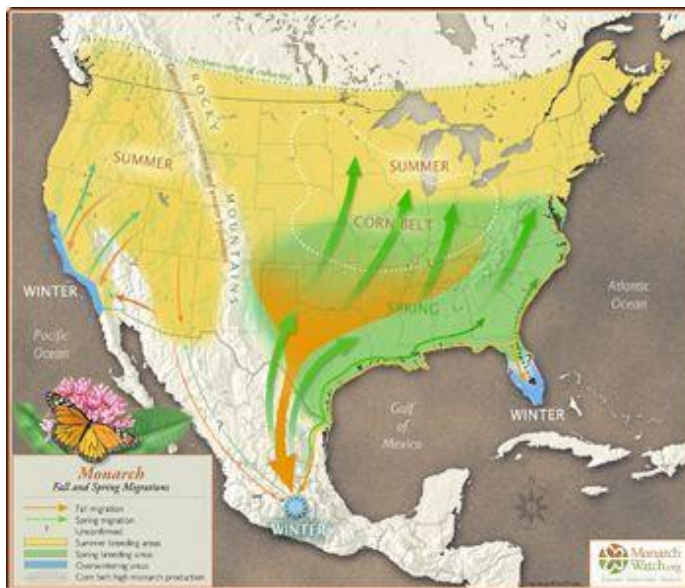


Figure 3. Monarch migration routes (Taylor 2010).

of the breeding range (Malcolm et al 1993, Howard and Davis 2004).

During the spring and summer breeding seasons, monarchs greatly increase the population size, reversing effects of population decline during fall migration and winter (Calvert 1999). The entire United States is repopulated within four generations before a fall generation starts southward migration to Mexico (Malcolm et al. 1993). Monarchs stop along the way to rest and nectar. There are two flyway patterns in the fall (Howard and Davis 2009), a Central flyway and an Eastern/Coastal flyway. These flyways together cover the most of Texas.

2.1.2. Spring Migration

In the spring, the first state occupied by migrating monarchs is almost always Texas (Howard and Davis 2004). After reaching Texas, assuming monarchs cannot cross the Gulf of Mexico, a portion of monarchs move towards eastern states before spreading northwards (Howard and Davis 2004). The timing of the spring migration varies inter-annually due to different biotic and abiotic factors (Howard and Davis 2004). Climatic and weather pattern can vary widely between years during the spring migration and development of the first generation (Zalucki and Rochester 2004, Feddema et al. 2004). For example, as females lay eggs on emerging milkweed along their spring migration, environmental conditions affecting host plant emergence may impact timing of the migration (Howard and Davis 2004). Further, timing of milkweed emergence affected by warmer temperatures at the coastal area may contribute to the early eastward movement of some migrants (Howard and Davis 2004).

Arrival of spring migrants in 1997-2002 at the latitudes of 30°-34° was on average on March 26, by the Journey North data (Howard and Davis 2004). They suggest a constant rate of migration throughout these lower latitudes. Further, they suggest that the primary factor affecting the timing of arrival is the starting date of migration at the overwintering sites. Oviposition occurs later than the arrival to an area (Howard and Davis 2004).

Monarchs appear to practically vacate Texas for the most of the summer (above latitude 35°). High temperatures and dieback of host plants have been suggested for the main contributing factors (Malcolm et al. 1987, Calvert 1999). In the fall, low densities of breeding monarchs have been observed to be present in Texas during September (Calvert 1999). The main mass of the fall migrating monarchs are not in Texas by the end of September. Timing of the monarch arrival to Texas in the fall is different along the Central and Coastal flyways (Howard and Davis 2009). In 2007, approximately after seven weeks of migration, roosting monarchs on the Central flyway were observed, on average, at latitude 34°, and on the Coastal flyway at 40°-42° (Journey North 2017b, Howard and Davis 2009).

2.1.3. Fifth Generation

Borland et al. (2004) examined the proportion of mated females collected throughout Texas from mid-September to late October. They found that about 60-80% of early migrants collected around 18-21 September were reproductive, compared to generally less than 40% of migrants being reproductive in late October. The main mass of migrants generally begins to reach north central Texas around 29 September (Calvert 1999, Monarch Watch 2017a, Journey North 2017b). Reproductive migrants in earlier September appear to be the origin of fifth generation larvae found in Texas by Calvert (1999), Prysby and Oberhauser (2004), and Batalden and Oberhauser (2015).

Batalden and Oberhauser (2015) collected several hundred roosting monarchs on 15 October about 240 km west of San Antonio and divided them into large outdoor cages with either no plants, *A. asperula* subsp. *capricornu* (antelopehorns), *A. oenotheroides* (zizotes), or *A. curassavica* (tropical milkweed). They found that about 7% of the males held in *A. curassavica* and *A. oenotheroides* cages mated within 11 days, and some eggs were produced from females. However, no mating or

oviposition was observed in cages with no plant or with *A. a. capricornu*. These results provide evidence that diapausing fall migrants can break diapause in the presence of host plants.

Calvert (1999) inventoried milkweed and monarch egg and larva along a Cross-Texas transect from Ozona through Austin to Pineville between 21 September and 18 November 1996. Above average fall precipitation likely contributed to the presence of *A. viridis* (green antelopehorns), *A. a. capricornu*, *A. oenotheroides*, and *A. latifolia* (broadleaf milkweed) along various parts of the transect. He found eggs (including those of the queen butterfly, *Danaus gilippus*) and larvae on all of these milkweed species along the eastern and western ends of the transect. The number of eggs and larvae declined in the western and eastern ends of the transect as the season progressed. Both eggs and larvae were found east of Austin in late September but not in early October or November. However, eggs and larvae were found west of Austin also from September 27 to 17 November. He suggested that the monarch breeding was already over in East Texas by the time the main migration mass arrived, but simultaneously continued in West Texas into November. He concluded that most of the fall breeding activities occurred west of Austin on *A. latifolia*.

Prysby and Oberhauser (2004) found evidence of a strong fall fifth generation of monarchs across Texas from late September to October on *A. curassavica* grown in gardens. Batalden and Oberhauser (2015) found a peak in monarch eggs around the second week of September at 30°N, which includes the south central Texas region, and they attributed this oviposition to early migrants.

Calvert (1999) found a notable absence of fifth generation eggs or larvae between 98° and 94.5°W, despite the abundance of milkweeds. Calvert (1999) suggested two explanations for the lack of monarch eggs and larvae in the east-central prairies (east of Austin): 1) fall migrating monarchs largely avoid these areas and only little oviposition occurs there, 2) predation by the introduced red imported fire ant (*Solenopsis invicta*) impacts the distribution of monarch eggs and larvae. The first suggestion corresponds with a potential low abundance of early migrants found between the fall Central and Coastal flyways and along their margins. Calvert (1999) suggested that predation by red imported fire ants best explains the spatial distribution of monarch eggs and larvae in 1996. Absence or low densities of red imported fire ants in the West and less dense single-queened colonies in the East may have contributed to higher survival rates. Long-term records show that abundance of the lepidopterans has decreased 50% from the pre-fire ant levels in the vicinity of Austin (Calvert 1999).

2.1.4. Fall Migration

The tagging of monarchs migrating from the regions extending from southern Canada to Texas down to the overwintering site of the high-elevation oyamel fir forests of central Mexico, within and surrounding the Monarch Butterfly Biosphere Reserve, has been monitored since 1992 by the Monarch Watch program (Monarch Watch 2017b). Analysis of this data is in progress, but preliminary results indicate that fall migration success is around 3 to 5 times higher from the center

of the corn belt between 90-95°W and 40-45°N than from all of the area east of 80°W (Taylor 2017). This trend is confirmed by previous studies finding that inland, non-coastal migrating monarchs are more successful than eastern coastal migrants in reaching the overwintering sites (Garland and Davis 2002, Brindza et al. 2008, Steffy 2015). Critical requirements for fall migrating monarchs include floral nectar for both the flight south and the building up of fat reserves for surviving the winter in Mexico (Brower et al. 2006, 2015), as well as appropriate roost sites for overnight shelter (Howard and Davis 2009). In the presence of southerly winds, monarchs roost in riparian areas sheltered from the wind in Texas and Mexico (Calvert 2001). In drier climates of Texas and Mexico, there is a general tendency for roosts to occur in cooler, moist riparian areas (Calvert 1998), such as the Dolan Falls Preserve on the Devils River (Aulbach 2005). Migrations routes in Trans-Pecos Texas and northern Mexico often follow riparian areas and ravines, and large roosts have been reported along the canyon walls of the Devils River near Del Rio, TX (Chip Taylor, Monarch Watch, personal communication).

2.1.5. Winter Breeding

Howard et al. (2010) documented winter resident monarch adults and larvae from central Texas to the area along the Gulf Coast and Florida. These habitats may attract monarchs due to favorable climatic conditions most years and year-round presence of *A. curassavica* (Batalden and Oberhauser 2015). The monarchs were not observed to form overwintering roosts, as they do at the Mexican overwintering sites (Howard et al. 2010). Batalden and Oberhauser (2015) conducted year round surveys for eggs on both native milkweeds and *A. curassavica* in the San Antonio area of Texas, and found that egg densities peaked on native milkweeds in May followed by a decline until September and October. But egg densities on *A. curassavica* remained steady from May to December, dropping significantly in January before beginning to rise again in February. Satterfield et al. (2015) found that winter-breeding monarchs in the southern US experience high OE infection levels and could serve as a source of infection for larger migrating populations in the spring and fall.

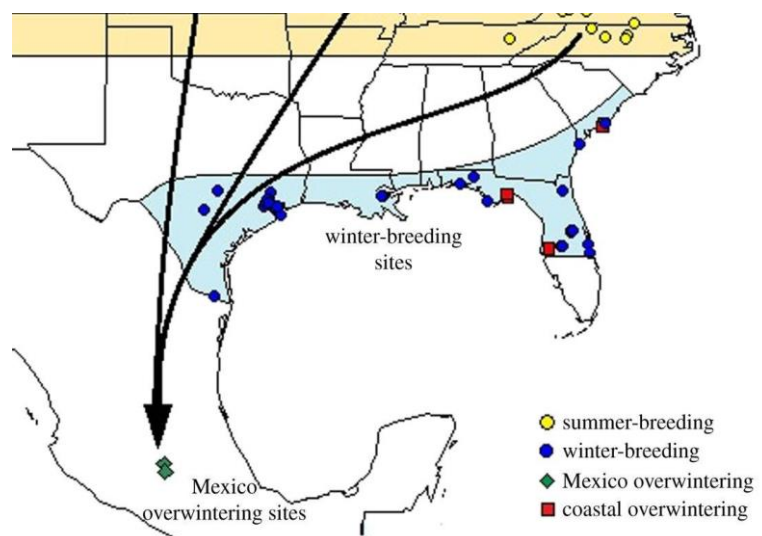


Figure 4. Observed winter breeding sites in southern US (modified from Satterfield et al. 2015).

2.2. Monarch Habitat Types

2.2.1. Land Use/ Land Cover

Monarchs are considered habitat generalists. They are dependent on habitats with milkweed and nectar sources. These can be found on various of different land use/ land cover categories, from grasslands to agricultural croplands and urban areas, such as road rights-of-way and gardens. However, there is still lack of deeper knowledge on quality and importance of different land use/ land cover categories as monarch habitats in Texas.

Nail et al. (2015) listed several factors that vary between habitats and may affect monarch survival. Heavily managed sites, such as gardens potentially have higher survival rate, if weed control and irrigation increase host plant quality. However, these sites may attract females, leading to increased immature monarch densities and density-dependent effects. Although, high egg density may indicate female preference for high quality host and also be associated with higher offspring survival. Monarch survival may decrease close to agriculture due to pesticide applications and other practices. Although these habitats may have lower abundance of predators supporting higher survival rate; more diverse habitats may support a more diverse and abundant suite of predators. Further, high egg density may contribute to higher disease rates and competition for resources, and thus, lower survival.

Rangelands

Rangelands in Texas and Oklahoma include land cover types of grassland/herbaceous and shrub/scrub, and these predominate over managed pastures (see below) in drier western parts of the states (Smeins, personal communication). Rangelands encompass several habitat types with varying suitability for milkweed, such as native grasslands, exotic grasslands, Conservation Reserve Program (CRP) lands, pasture, and grasslands converted from former croplands or pastures. Currently, based on geographic information systems (GIS) grasslands cannot be divided accurately into different subcategories (Pleasants 2017).

Pleasants (2017) estimated that, in 2014, most milkweed in the Midwest could be found in CRP lands (47%) and roadsides (34%). Several *Asclepias* spp. occurring in Texas and Oklahoma rangelands are known as poisonous to livestock, including *A. a. asperula*, *A. a. capricornu*, *A. latifolia*, *A. subverticillata* (horsetail milkweed), *A. verticillata* (whorled milkweed), and others are suspected as poisonous to livestock, including *A. viridis* (Stephens 1980, Hart et al. 2000). Consequently, ranchers may spot treat these milkweeds with herbicides in rangelands. Milkweeds in rangelands may also be exposed to herbicides from brush control treatments (Smeins, personal communication).

Managed Pastures

Permanent managed pastures encompass the pasture/hay land cover type. They are generally established with deep cultivation to eliminate weeds and prepare a seedbed (Dorsett and Householder 1986). The exotic warm season perennial grasses bermudagrass (*Cynodon dactylon*) and, to a lesser extent, bahiagrass (*Paspalum notatum*) are commonly grown in permanent managed pastures of Texas and Oklahoma (Dorsett and Householder 1986, Dorsett 1987, Hays et al. 2005, Oklahoma State University 2017). Several varieties of bermudagrass (*Cynodon dactylon*) are used for either grazing or hay in the central and eastern portions of both Texas (Corriher and Redmon 2011; Smeins, personal communication) and Oklahoma (Redfearn and Wu 2013, Arnall and Redfearn 2011), and irrigated bermudagrass pasture is increasing in the western Texas and Oklahoma panhandles (Redfearn and Wu 2013). A number of varieties of bahiagrass (*Paspalum notatum*) are utilized primarily for grazing in managed pastures of eastern Texas (Redmon 2001) and southeastern Oklahoma (Arnall and Redfearn 2011). Several *Asclepias* spp. may occur in Texas and Oklahoma pastures, several of which are known as poisonous to livestock, including *A. a. capricornu* to the east, and *A. a. asperula*, *A. latifolia*, *A. subverticillata* (horsetail milkweed), and *A. verticillata* (whorled milkweed) to the west. Other more eastern pasture milkweeds are suspected as poisonous, including *A. viridis* and *A. incarnata* (swamp milkweed) (Stephens 1980, Hart et al. 2000). Milkweeds are avoided as browse due their bitter taste, and they are generally only eaten in situations of overgrazing or drought (Hart et al. 2000). Horses are especially sensitive to milkweed poisoning from feeding on baled hay contaminated with thin-leaved milkweeds, such as *A. subverticillata* (Turner et al. 2015). Consequently, herbicides are suggested for control of *Asclepias* in grazing and hay pastures, especially where milkweeds are most likely to persist along field edges, fence lines, irrigation ditches, and roadsides (Baumann 2004, Turner et al. 2015), and use of the selective broadleaf herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is common (Smeins, personal communication). However, milkweeds are often tolerant to herbicide treatments and they may persist with stunted growth or return another season (Smeins, personal communication). The suitability of herbicide treated *Asclepias* as monarch larval host plants in managed pastures is probably severely limited. In addition, the thick cover of bermudagrass and bahiagrass pastures often limits establishment and growth of forbs (Hays et al. 2005), including *Asclepias* spp. (Smeins, personal communication).

Rights-of-Way and Urban Areas

Milkweed species in Texas are often associated with roadsides and railways (Woodson 1954), and probably also utility rights-of-way (Rager et al. 2013). And these rights-of-ways can also serve as habitat for nectar plants used by monarchs (Rager et al. 2013). Roadsides may be an important source of monarch breeding in the spring. Roadsides occupy a large portion of Texas. These areas can be considered as habitats for many species. Roadsides providing milkweed and nectar sources can be important habitats for monarchs along the migration route and their importance may even increase with the documented loss of milkweed within the breeding range (Mueller and Baum

2014).

Croplands

Milkweed abundance in the Midwestern row crops have been a main focus of study. Estimates for the current status of milkweed in crop fields vary. For example, Flockhart et al. (2015) proposed that, in 2013, in Central and North regions of the monarch distribution, a total of 2.7 billion milkweed stems (70%) could be found in corn and soybean fields. In contrast, Pleasants (2017) estimated the number of milkweed stems in the Midwestern corn and soybean fields (in 2014), to be 6 million stems (0.4%).

The range of *A. syriaca* (common milkweed), a common rhizomatous milkweed of Midwest croplands, does not extend south to Texas (Singhurst et al. 2015), and milkweed species in Texas are not observed to grow in crop fields (J. Singhurst, Texas Parks and Wildlife, personal communication). However, *A. latifolia* commonly grew in cotton fields of the Texas panhandle, such as around Bailey County, in the 1960's to 1970's before the increased use of glyphosate resistant cotton (S. Feagley, Soil and Crop Sciences Department, TAMU, personal communication). The use of herbicides in row crops does not appear to have impacted Texas milkweed abundance as much as in the Midwest, but herbicide applications around crop borders, rangelands, pastures, rights-of-way, and urban areas probably do limit milkweed abundance in Texas.

2.2.2. Roosting Habitats

Results of a study by Davis et al. (2012) indicate that almost 98% of the roosting during the fall migration occurred in trees. The rest of the roosting monarchs were observed on herbaceous vegetation. They did not find any preference in tree species. However, the most frequently reported trees species were pines and other conifers, maples, oaks, pecans, and willows (Davis et al. 2012). The frequency of used tree species as roosting sites varied longitudinally. Conifers and maples were used most often in the northern areas, and pecans and oaks in the southern parts of the flyway. The land use category associated with about half of the roosting sites was cropland. However, they found a shift in relative proportions of land use in Texas. In northern Texas (above 34°), 67% of the observed roosting sites were on croplands. In contrast, in southern Texas, 61% of the roosting sites were on grassland. Although, this shift may be related to available land cover across Texas rather than monarch preference. Davis et al. (2012) compared large-scale land use between monarch roosting sites with random locations. They suggested that the sites selected by monarchs had less overall deciduous forest cover, more urban area, a higher percentage of open water nearby, and less wetland cover than the random locations. However, they acknowledged bias in observations; participants tend to live in urban areas and may not look for roosts in densely forested areas. Roosting sites maybe also more difficult to see within forest canopy (Davis et al. 2012).

2.3. Milkweed Habitats in Texas

2.3.1. Overview

The primary host plants of monarch larvae are milkweeds of the genus *Asclepias*, of which 33 of the 108 known species in North America are documented to be used by monarchs (Ackery and Vane-Wright 1984, Malcolm and Brower 1986, Lynch and Martin 1993). There are few published reports of milkweed host use in Texas. We first discuss milkweed use of monarchs in Texas in reference to the three monarch phenophases of spring first generation, fall fifth generation, and winter-breeding. We then discuss habitat for the primary milkweed host species (based on current information) and the importance of different habitat types in Texas.

2.3.2. Seasonal Use of Milkweeds by Monarchs

Spring First Generation

The three native milkweed species most commonly reported from Journey North (2017a) and the Monarch Larva Monitoring Project (MLMP 2017) for the spring in Texas include *A. a. capricornu*, *A. viridis*, and *A. oenotheroides*. Calvert (1996) reported monarch eggs and larvae on *A. oenotheroides* from 29 March to 7 April, 1995 in south central Texas in a pasture near Luling. All three of these species are herbaceous perennials blooming mostly from spring to summer (Woodson 1954). The US Fish and Wildlife Service (Tuggle 2014) has identified these three milkweeds as the most important for monarchs in Texas, and the USDA NRCS (2015a) has targeted these species for conservation efforts in their south central subregion which includes Texas, Oklahoma and Kansas. Lynch and Martin (1993) monitored monarch larvae through the fifth instar on *A. amplexicaulis* (clasping or blunt-leaf milkweed) and *A. viridiflora* (green comet milkweed) from 19 April to 7 May 1985 one mile east of Ore City in northeast Texas. Over a similar time period, they also monitored larval populations on *A. viridis* at two locations in Texas and two in Louisiana and *A. a. capricornu* at two locations in Texas, and also found that fifth instars were successfully produced by early May at most locations. Additional milkweeds used in the spring in natural areas of Texas include *Asclepias incarnata*, *A. texana* (Texas milkweed) (Berman and Baum unpublished), and *A. tuberosa* (iNaturalist 2017b). Milkweeds reported as used by monarch larvae in Texas home garden settings during the spring or summer include *A. angustifolia* (Arizona milkweed), *A. curassavica*, *A. fascicularis* (California narrowleaf milkweed), *A. incarnata*, *A. syriaca*, *A. texana*, and *A. tuberosa* (Journey North 2017a, iNaturalist 2017b).

Fall Fifth Generation

Calvert (1999) surveyed roadsides along an 800 mile east/west transect extending from Jasper to Ozona during 27 September to 13 November, 1996. He reported that above average rainfall produced flowering in four species of milkweed *Asclepias viridis*, *A. a. capricornu*, *A.*

oenotheroides, and *A. latifolia* (in order of abundance). He found the greatest abundance of monarch eggs and larvae in the fall on *A. latifolia*, followed by *A. a. capricornu*, *A. viridis*, and *A. oenotheroides*. The number of eggs and monarch larvae on *A. latifolia* was three times higher than on *A. viridis* and two times higher than on *A. a. capricornu*. Milkweed species were distributed continuously throughout the transect with some exceptions. He found that almost always one milkweed species occupied each roadside patch. In rare occasions, two species were found within one patch. For example, *A. latifolia* and *A. a. capricornu* were present in the same area in West Texas, and *A. oenotheroides* occurred with *A. viridis* in East-Central Texas. Calvert (1999) suggested that because of this spatial separation of milkweed species, the oviposition data may not show monarch preferences among the milkweed species, but more likely reflect the presence of certain species within a certain geographic area. Batalden and Oberhauser (2015) found a preference for monarch oviposition on *A. curassavica* over *A. a. capricornu* in the fall (but not spring) where both species were present together in the vicinity of San Antonio, Texas. Additional milkweeds reported as used by monarchs in Texas during the fall include *A. incarnata* and *A. perennis* (aquatic milkweed) (Berman and Baum, unpublished data).

Winter Breeding

Home garden plantings of *A. curassavica* are the most common milkweed available and used by monarch larvae through the winter in south-central and coastal Texas (Batalden and Oberhauser 2015). The seasonal and regional availability of *A. curassavica* in the south central US is further discussed below.

2.3.2. Abundance and distribution of common Milkweed Hosts in Texas

Overview

As detailed above, *A. viridis*, *A. a. capricornu*, and *A. oenotheroides* are the most abundant spring host plants for first generation monarch larvae in Texas based on currently available data. *Asclepias latifolia* is an important fifth generation host plant in west Texas. *Asclepias curassavica* is an introduced ornamental commonly utilized by all generations, especially for the fall fifth generation and most winter breeding monarchs in Texas. We detail the Texas habitats of these four focal milkweed species below, and include their distribution in the south central states of Texas, Oklahoma, Arkansas, and Louisiana (only data for *A. viridis* has yet been incorporated from Global Biodiversity Information Facility [GBIF 2017], and from georeferencing location descriptions in records from the Oklahoma Biological Survey [OBS 2017]).

Asclepias viridis (Green Antelopehorns)

Calvert (1999) found that the most abundant milkweed species in central Texas, *A. viridis*, is most common east of 98°W (Fig. 5). This species is common in glades (grasslands interspersed among woodlands) and prairies from eastern Texas north to eastern Kansas and in Missouri (Woodson 1954). In central Texas, it occurs on sandy soils (Reeves 1972), and along the Texas coastal plains it has been noted on sandy or clayey prairies and glades and roadsides (Lehman et al. 2005). It also occurs on dry hillsides, dry pine barrens, and is especially abundant in overgrazed pastures, roadsides, and disturbed ground in Texas (Singhurst et al. 2015). *Asclepias viridis* has also been reported from gravelly and rocky granite and limestone habitats (OBS 2017).

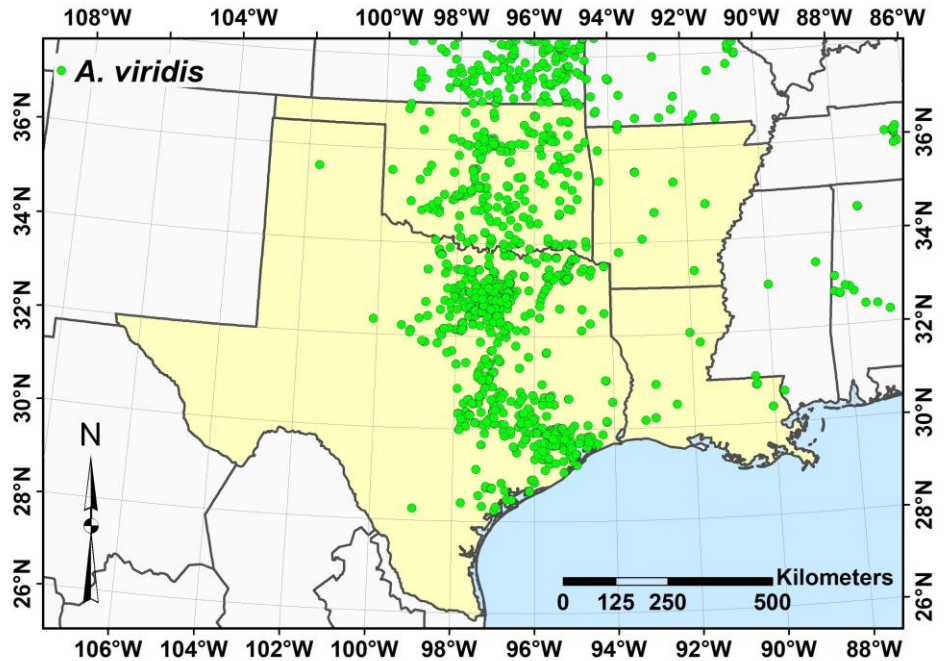


Figure 5. Distribution of *Asclepias viridis* in south central US (GBIF 2017, OBS 2017, SeiNET 2017, iNaturalist 2017a).

Asclepias asperula subsp. *capricornu* (Antelopehorns)

The subspecies *A. a. capricornu* is the most abundant milkweed found along the eastern Edwards Plateau and Cross Timbers of central west Texas, and it is the predominant subspecies of *A. asperula* east of -102°W (Woodson 1954) (Figs. 6-7). The subspecies *A. a. asperula* (spider milkweed) is heavily predominant west of -102°W in the US and in Mexico (Woodson 1954). Singhurst *et al.* (2015) suggest that species status for *A. a. capricornu* may be warranted due to differences in the morphology and distribution of the two subspecies.

Asclepias a. capricornu is found in prairies, limestone, sandstone, clay hills, and pastures (Woodson 1954). This subspecies is also noted as occurring on rocky and sandy prairies in general (Singhurst *et al.* 2015), on dry soils in central Texas (as *A. decumbens*, Reeves 1972), and on caliche in Goliad County along the coast (Lehman *et al.* 2005). The subspecies *A. a. asperula* can be found along arroyos and draws in west Texas (Hart *et al.* 2000).

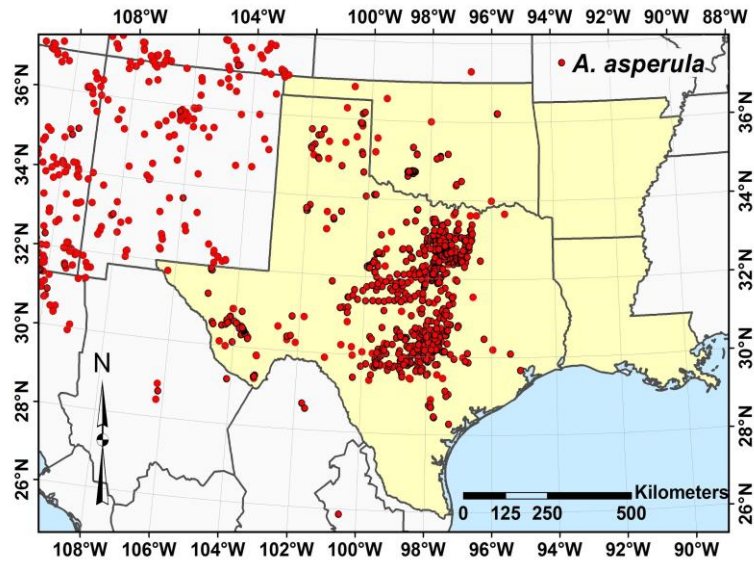


Figure 6. Distribution of *Asclepias asperula* in south central US (SeiNET 2017, iNaturalist 2017a).

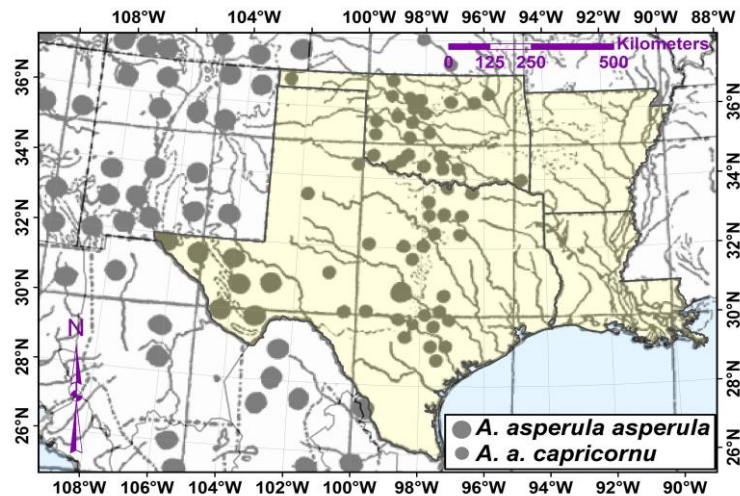


Figure 7. Distribution of *Asclepias asperula asperula*

Asclepias oenotheroides (Zizotes)

Asclepias oenotheroides is probably the most abundant milkweed in southern Texas, but it is most often reported from the southeastern portion of central Texas. It is widely distributed across the state, with the exception of the eastern edge, where it is notably absent in the South Central Plains ecoregion (iNaturalist 2017a, SEINet 2017) (Fig. 8). It is generally found on rocky, clay soils of llanos, mesas, and hills, as well as fields, thickets and roadsides (Woodson 1954). Along the Texas coast, it is found in prairies, openings, waste places, and commonly on island dunes (Lehman et al. 2005). Singhurst et al. (2015) notes it occurring on rocky calcareous limestone and igneous soils in the interior portion of the state, as well as in sandy clay soils and dunes along the coast.

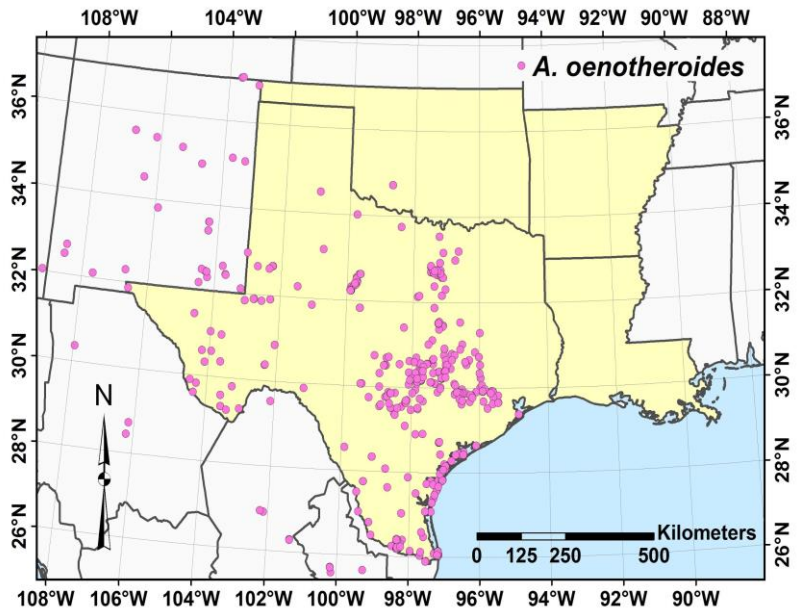


Figure 8. Distribution of *Asclepias oenotheroides* in south central US (SeiNET 2017, iNaturalist 2017a).

Asclepias latifolia (Broadleaf Milkweed)

Asclepias latifolia is both restricted to, and frequent to abundant within, the western portion of Texas, west of the Cross Timbers and eastern Edwards plateau (Woodson 1954, Hart et al. 2000, iNaturalist 2017a, SEINet 2017) (Fig. 9). Woodson (1954) notes it as occurring in mixed prairies, badlands, high plains, roadways, and railways. Hart et al. (2000) reports it as common along trails and roadsides, but less common in pastures, and Stephens (1980) lists it from dry, sandy soils. Singhurst et al. (2015) lists dry plains, prairies, and breaks on sandy, clayey, or rocky calcareous soils as habitat for *A. latifolia* in Texas.

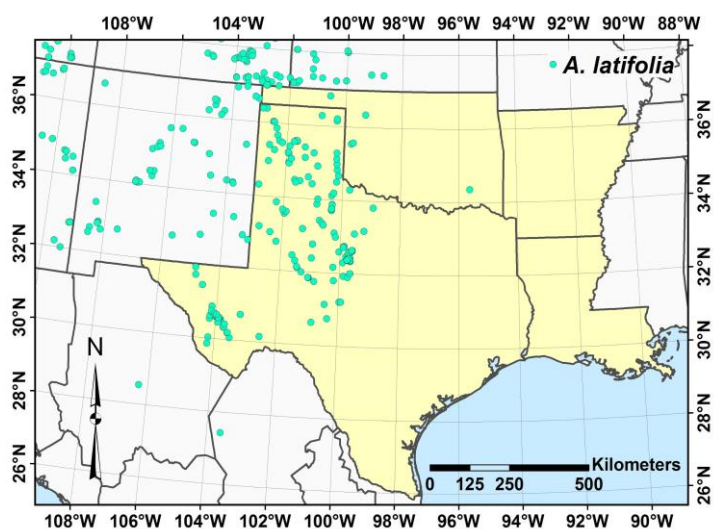


Figure 9. Distribution of *Asclepias latifolia* in south central US (GBIF 2017, SeiNET 2017, iNaturalist 2017a).

Asclepias curassavica (Tropical Milkweed)

Asclepias curassavica is an introduced herbaceous perennial that is locally naturalized in parts of southeast Texas and extreme south Texas in Cameron and Hidalgo Counties (Fig. 10) (Singhurst et al. 2015). It is widely cultivated across the state in home gardens to attract butterflies (Singhurst et al. 2015, Journey North 2017a). *Asclepias curassavica* is evergreen, generally surviving the winter with leaves and sometimes blooms, in USDA Plant Hardiness Zone (PHZ; USDA Agricultural Research Service 2012) 9b (south Texas and edge of Texas coast). It is hardy, generally surviving the winter to resprout from the roots, in PHZs 9a (Houston) to 8b (San Antonio and Austin) (Mahr 2008, Journey North 2017a). It can sometimes reseed and persist as an annual as far north as PHZ 7 (Oklahoma City, Tulsa) (Winter 2003). In some, probably milder winters, there are several reports of *A. curassavica* with persistent leaves and even blooms during winter in PHZ 9a from the Houston area and coastal Louisiana (New Orleans and Metairie) (Journey North 2017a).

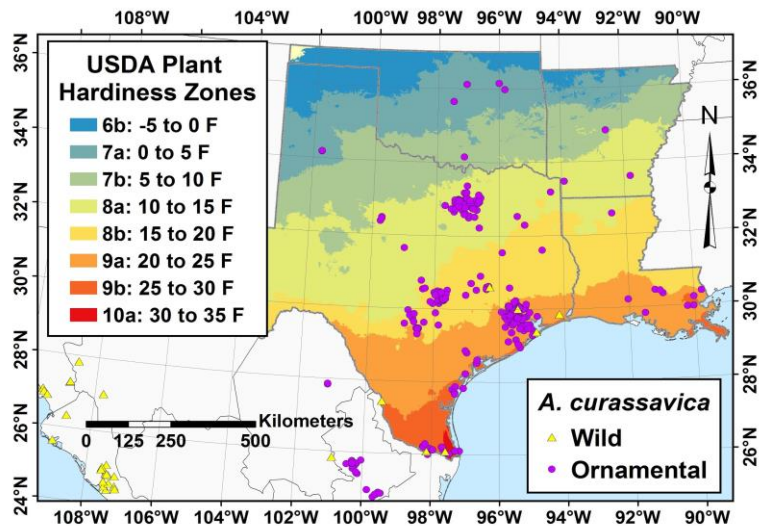


Figure 10. Distribution of ornamental and wild *Asclepias curassavica* in south central US (ornamental: Journey North 2017a, MLMP 2017,

2.4. Nectar Resources in Texas

An important factor for monarchs is adequate nectar sources along the migration route to ensure successful overwintering. Monarchs obtain sugars from nectar plants which are converted into lipids. It is thought that the fall migration itself may not be energetically costly when winds are favorable (i.e., soaring is not energetically costly), and that monarchs accumulate lipids during fall migration for energy sources for the overwintering period (Brower et al. 2006), and possibly for the spring migration (Brower 1985). The degree of nectaring during spring migration is not well known (Brower et al. 2006). Urquhart (1960) suggested that monarchs rarely nectar in the Spring. However, Brower (1995) stated that the assumption was based on limited data and incomplete understanding of the monarch migration. The summer breeding generations feed on nectar plants and maintain low lipid levels during the time they are dispersing, mating, and ovipositing (Brower et al. 2006).

Monarchs may change their behavior and increase their rate and duration of nectaring when approaching overwintering sites, as in Texas and northern Mexico (Brower et al. 2006). This pattern suggests that the extensive lipid deposition is for overwintering, not for the journey (Brower et al. 2006). According to Brower (1985), monarchs accumulate most of the lipids in Texas, and North and Central Mexico. Brower et al. (2006) disagreed with the results of the study by Brower (1985) that monarchs accumulate few lipids before reaching Texas. However, they agreed that the accumulation was much less in Virginia than in Texas. Brower et al. (2006) suggested that within Central and Coastal flyways in Texas and North Mexico, some specific locations may have key importance for monarch nectaring. This contrasts with most of the migration route, where specific stopover sites do not seem to be used by a large number of migrants.

Monarchs do not display nectaring or habitat specialization during fall migration (Brower et al. 2006). Monarchs nectar on dozens of flower species (Robertson 1928, in Tooker and others 2002) in almost any open habitat. Monarchs can be seen nectaring in large numbers where nectar plants are abundant, such as in fields of goldenrod and other asters, along streams, and nonherbicide roadways (Brower et al. 2006). Some agricultural crops also provide nectar sources to monarchs, including red clover and alfalfa (Brower et al. 2006). Even some invasive plant species, such as purple loosestrife (*Lythrum salicaria*), can provide significant nectar sources to monarchs, in areas where poorly controlled (Brower et al. 2006). They note that invasion control within those areas may even limit the nectar sources available to monarchs depending on the availability of other flowering plant species.

Common very high value monarch nectar plants in the fall migration for Texas include *Verbesina encelioides* (golden crownbeard), *Baccharis neglecta* (Rooseveltweed), and *Conoclinium coelestinum* (blue mistflower) (USDA NRCS 2015b). The importance of different nectar plants may vary among years based on flowering phenology and the timing and duration of the fall migration (K. Baum, personal observation).

3. Threats

3.1. Overall Threats

Three major factors have been identified in the decline of the eastern migratory population (e.g., Flockhart et al. 2015, Jepsen et al. 2015): (1) loss of milkweed habitat in the US related to increased use of herbicides on herbicide resistant crops and land use change (e.g., Brower et al. 2002, Zaluki and Lammers 2010, Brower et al. 2012, Vidal et al. 2014, Pleasants 2017), (2) deforestation of the oyamel fir overwintering sites in Mexico (Brower et al. 2012, Vidal et al. 2014, Brower et al. 2016), and (3) climate change with related extreme weather events, such as droughts and more intense winter storms (Oberhauser and Peterson 2003, Brower et al. 2012, Howard and Davis 2015). Additional potential factors in their decline include insecticide exposure (e.g., Krischik et al. 2015), natural enemies (e.g., Prysby 2004, Rayor 2004, Sternberg et al. 2013), and road

mortality during the fall migration (McKenna et al. 2001).

Factor affecting the decline of the monarch can be divided among the seasonal phases of their annual cycle (Inamine et al. 2016). We phenologically divide threats to the eastern migratory population of monarchs among four main phenophases for Texas: (1) spring migration; (2) fifth generation; (3) fall migration, and (4) winter breeding. Below, we discuss factors in the decline of monarchs for these phenophases, emphasizing our focal region of Texas.

3.2. Spring Migration

The exposure to cold weather over the course of the winter at the overwintering site triggers monarchs towards a northern flight orientation for the spring migration (Guerra and Reppert 2013). Adults migrating north oviposit first generation eggs on milkweeds primarily in the area of Texas and Oklahoma (Flockhart et al. 2013). Quality and phenology of milkweed vary spatially, and female monarchs may choose not to lay eggs at all places they stop (Howard and Davis 2004). Healthy populations of milkweeds in an environment without damaging levels of pesticides are critical to larval development. Pleasant and Oberhauser (2013) and Flockhart et al. (2015 and 2017) suggest the Upper Midwest is a critical area for later generations of breeding. The average portion of monarchs with Midwestern origin (i.e., developed as caterpillars in the Midwest) was 38% during past 40 years (Flockhart et al 2017). Studies suggest that over half of the monarchs successfully reaching overwintering sites originate outside of the agricultural Midwest (Hobson 2008, Flockhart et al. 2013, 2017). On average, 11% of the overwintering monarch are from southwestern portion, which includes Texas (Flockhart et al 2017).

Prevailing conditions at the overwintering sites may also impact the spring remigration. For example, Brower et al. (2016) reported illegal logging of oyamel fir forest in Arroyo Hondo, Sierra Chincua (19°40'N, 100°18'W), occurring in summer 2015 within an area of approximately 10 hectares. This area is important for monarchs, and Brower et al. (2016) raised a concern for future colonies overwintering in the area. Monarchs move downslope as the overwintering season advances (Brower et al. 2016). They have tend to reach down to the area, now clear cut, in mid-March and started the migration to north shortly after (Brower et al. 2016). They proposed that it is possible that the monarchs in the area, may start migration prematurely and fail to recolonize the Gulf Coast states or they may be forced into forested areas with less microclimatic protection.

3.2.1. Monarch Habitats

Land Use/ Land Cover Change

Deep tillage and plowing of row crops apparently prevents establishment of most milkweed species in Texas, with the previously noted exception of *A. latifolia* in the panhandle. In the Midwest, *A. syriaca* spreads extensively by rhizomes and is common in row crop fields (Yenish Literature Review

et al. 1997, Hartzler 2010, Pleasants and Oberhauser 2013, Zaya et al. 2017), where it has served as an important resource for monarch larvae. As early as 1954, Woodson (1954) noted the decline of *A. viridis* in parts of southern Ohio due to habitat loss from cultivation. In pasture/hay plots in Texas, milkweeds can persist if cultivation is minimal, but they may be stunted or killed by herbicide treatments (F. Smeins, personal communication).

Loss of monarch habitat may occur when habitats with milkweed or nectar plants are converted into other land use categories that may not support these species, such as croplands in Texas. Lark et al. (2015) conducted a four-year spatially explicit analysis of changes between cropland and non-cropland in the conterminous USA. They found that total net cropland area increased by over 12,000 km² between 2008 and 2012. Almost 30,000 km² of uncultivated land (land use status since 2001) were converted to cropland during the four years (Lark et al. 2015). Some 18,000 km² were removed from production, of which, up to 85% was enrolled into the Conservation Reserve Program (CRP). They found significant geographical variation in the cropland adaptation, and cropland expansion mainly occurred east of the Missouri river and within the northern Prairie Pothole Region. They also found highly concentrated hot spots of cropland expansion in the Panhandles of Texas and Oklahoma (Figure 11). The main land use categories converted to cropland were native and planted grasslands, followed by shrubland and idle land (Lark et al. 2015). Approximately 6,500 km² of long-term unimproved grassland were altered to cropland; most of it occurred in the Central Plains from North Dakota to Texas. The most common crops planted first after the conversion, were winter wheat and cotton in northern Texas and oats, sorghum, and rice in central- and south-Texas (Lark et al. 2015). These results are in accordance with national findings of Faber et al. (2012). According to their report, 9,300 km² of grassland, shrubland, and wetland was converted into croplands in the US during 2008-2011. Some of the highest rates of the habitat conversion occurred in drought affected parts of West Texas and Oklahoma. Within ten west Texas counties, more than 2650 km² of wildlife habitat were converted in order to plant cotton, corn, and wheat (Faber et al. 2012).

Herbicides and Milkweed Loss

Most of the milkweed loss in the Midwest has occurred in cropland, especially in corn and soybean fields (Pleasants 2017). With the advent of genetically modified glyphosate resistant crops in 1999,

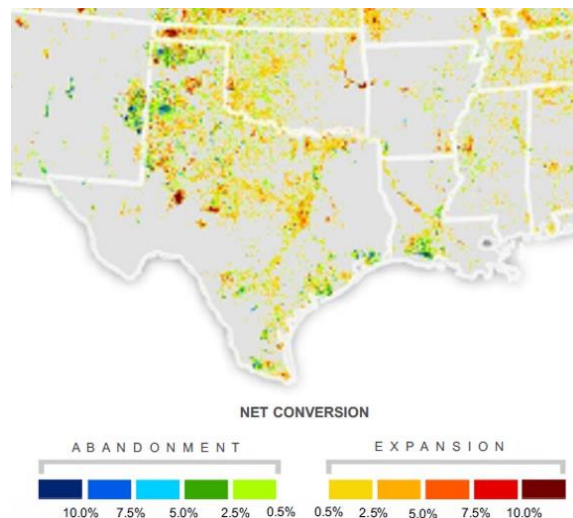


Figure 11. Net conversion to and from cropland in 2008-2012. Modified from Lark et al. 2015. © IOP Publishing Ltd.

the frequency of *A. syriaca* declined in Iowa croplands from 50% to 8% and its area in cropland dropped by 90% from 1999 to 2009 (Hartzler 2010). Pleasant and Oberhauser (2013) conclude that a 58% decline in *A. syriaca* due to glyphosate resistant crops from 1999 to 2010 is a major driver in the 81% decline in monarchs in the Midwest over this same period, and the decline is also correlated with the reduction in the Mexican overwintering population. Pleasants (2017) estimated a total loss of over 11 billion milkweed stems in the Midwest in 2008-2014 combining milkweed loss in corn and soybean fields with loss due to land use change. These findings are further supported by Zaluki and Lammers (2010) and Stenoien et al. (2015). Although total usage of pesticides in Texas has remained almost constant from 1992 to 2014 (NAWQA project 2017), the use of glyphosate has been increasing almost exponentially during the same period, from approximately 500 metric tons in 1992 to over 8,100 metric tons in 2014 (NAWQA project 2017). Glyphosate use on herbicide resistant cotton may have contributed to the elimination of the once common occurrence of *A. latifolia* in croplands of the Texas panhandle (S. Feagley, personal communication).

Pleasants (2017) mentioned two potential reasons why milkweed loss translates into reduced monarch production. One hypothesis is that female monarchs are forced to lay more eggs per milkweed stem. According to Nail et al. (2015), monarch survival to the adult stage is lower with higher egg density. However, the number of monarch eggs per stem has not increased since 1999 (Pleasants 2017). On contrary, the egg density may have decreased (Stenoien et al. 2015). The other potential factor by Pleasants (2017) is increased difficulty for females to find milkweed along decreased milkweed abundance.

Insecticides

Larval monarchs often feed on milkweed in or near agroecosystems where they may be exposed to pesticides (Pecenka and Lundgren 2015). Adult monarchs may also be exposed to pesticides around croplands during spring migration. Pollinator exposure to pesticides, especially honey bees, has increased in interest among scientists in recent years. However, pesticides as a threat to specifically monarchs have not been widely studied.

Of the insecticides affecting pollinators, neonicotinoids have received the most attention (Krupke et al. 2012). According to Goulson (2013), neonicotinoid insecticides have become the most widely used pesticides worldwide. These relatively recently introduced chemicals are heavily applied in both agricultural and urban areas (Krupke et al. 2012). Neonicotinoids are persistent and the half-lives can be measured in months (US Environmental protection Agency 2003). Neonicotinoids are water-soluble and systemic, being transported throughout developing plants. Untargeted plants nearby or among treated crops may take up excess neonicotinoids (Krupke et al. 2012). Pecenka and Lundgren (2015) studied non-target effects of clothianidin (a neonicotinoid) on immature monarchs. Their experiment revealed sub-lethal effects on monarch larvae. Sub-lethal effects included decreased body weight and length and head capsule width. Their toxicity

assessment indicated also lethal effects on young monarch larvae. One of the major use for neonicotinoids is applications to maize seed (Krupke et al. 2012). Pecenca and Lungren (2015) conclude that, within the Corn Belt, monarch larvae are exposed to potentially harmful doses of clothianidin. They suggest that more research needs to be done within wider geographic and seasonal ranges to determine if the insecticide is contributing to the monarch decline. According to Krupke et al. (2012), bees are exposed to neonicotinoids in and near agricultural fields throughout the spring and summer. The highest risk for exposure is during and after maize planting due to high concentration of neonicotinoids in talc exhausted to the environment. Talc is commonly added into corn seed boxes in order to prevent friction and stickiness and much of it is exhausted during the planting (Krupke et al. 2012). In 2016, there were approximately 12,000 km² of corn fields in Texas (CropScape 2017). The usual planting dates of corn in Texas range from early March till mid-May (USDA 2010), which overlaps with monarch spring migration. Monarchs nectar in alfalfa and red clover fields (Brower et al. 2006), but alfalfa is typically cut before flowering in Texas to reduce infestation of blister beetles (C. Allen, Texas AgriLife Extension, San Angelo, Texas). Monarchs also nectar in canola in the spring (McCoshum and Baum 2014) and the potential for their exposure to insecticides in canola requires further consideration.

Rangelands

Altered Fire Regimes and Woody Encroachment

Historical fire regimes in the Great Plains were important for maintaining diversity of both plant and animal species (Harrison et al. 2001), and are associated with preventing the currently observed woody invasion of grasslands that can lead to reduce prairie forb diversity (Van Auken 2009, Ratajczak et al. 2014). Woodson (1954) noted the loss of grassland habitat for *A. viridis* in southern Ohio due to woody encroachment. Summer burns can stimulate late season growth of *A. viridis* (including flowering) that are utilized by fall fifth generation monarchs in the Great Plains (Baum and Sharber 2012). Further evidence of fire adaptation in *Asclepias* is seen in the enhanced germination of *A. syriaca* seeds when stratified with an aqueous smoke solution (Mojzes and Kalapos 2015).

Overgrazing

Milkweeds of the Great Plains historically flourished under patchwork grazing by bison (USDA NRCS 2016). An appropriate level of cattle grazing can be critical in maintaining grassland biodiversity, benefiting a variety of forbs by reducing competition with grasses, including milkweeds, which are generally less palatable for cattle. Towne et al. (2005) investigated effects of grazing in tallgrass prairie vegetation in a 10-year experimental study. Abundance of perennial forbs increased, including known good nectar sources for monarchs, in grazed pastures compared

to ungrazed control sites. Increase of the forb cover was greater after bison grazing than cattle grazing. Towne et al. (2005) proposed that land management practices within the Great Plains have a significant effect on nectar plant abundance and diversity. However, overgrazing can severely reduce the abundance of a variety of forbs, including milkweeds. Overgrazing also reduces fine fuels needed for prescribed fire and can increase susceptibility to woody plant invasion. Some nectar plants are also very palatable to livestock (sometimes referred to as ice cream plants), and may not occur in grazed sites.

Invasive Plant Species

Invasive plants, especially grasses, have contributed to the reduction of biodiversity in Great Plains grassland ecosystems (Hickman et al. 2006). The cover of various native grasses and forbs, including *A. viridis*, were reduced in Oklahoma tallgrass prairie invaded by King Ranch (KR) bluestem (*Botriochloa ischaemum* var. *songarica*), but increased following suppression of KR bluestem with sustained late season mowing (Dee et al. 2016). King Ranch bluestem is also widely invasive along roadsides and grasslands of central and southern Texas, and it contributes to decreased floral diversity in the eastern Edwards Plateau (Gabbard and Fowler 2007), where it potentially may suppress *A. a. capricornu* and *A. oenotheroides*. Other invasive grasses, such as Kleberg bluestem (*Andropogon annulatus*), may also suppress milkweeds in roadside and pasture environments of Texas (C. Best, USFWS, Austin, Texas, personal communication).

3.3. Fifth Generation

Fall migrating monarchs produce a fifth generation on *A. latifolia* and sometimes available late season milkweed resprouts of *A. a. capricornu* and *A. viridis* in Texas and Oklahoma (Calvert 1999, Borland et al. 2004, Mueller and Baum 2014). These milkweeds are adapted to historic grassland disturbance regimes of summer bison grazing and wildfires (NRCS 2016), which could have potentially produced locally abundant late season resprouts of milkweed (Mueller and Baum 2014, Fischer et al. 2015). The size of the fifth generation likely varies among years, and in some years may be important in buffering monarch populations against declines caused by factors present on their spring and summer breeding grounds, such as drought or fire. On average, the fifth generation from the region including Texas and Oklahoma contributes 11% of the monarchs present on the overwintering grounds, but this percent can be much higher in some years (Flockhart et al. 2017). The potential loss of periodic summer disturbance related large populations of fifth generation adults may be reducing the resilience of the monarch population to decline from seasonal extreme events.

3.4. Fall Migration

3.4.1. Overview

Ries et al. (2015) analyzed trends in North American Butterfly Association's (NABA) adult eastern migratory monarch population data during the breeding season and the area of occupied overwintering habitat in Mexico. They detected the downward trend in overwintering area size, but not in the summer breeding population, indicating a potential decline in the population following summer breeding during the migration. They hypothesized that one potential mechanism in fall migration losses could be an increase in the number of migrating adults dropping out of diapause to oviposit on increasingly available home garden plantings of exotic *A. curassavica* in the South (Batalden and Oberhauser 2015), which can lead to an increase in the buildup of the OE parasite (Satterfield et al. 2015).

Badgett and Davis (2015) found no evidence of decline in summer breeding populations of monarchs in northern Michigan, and suggested that factors impacting the decline of monarchs during overwintering and fall migration are more important than declines during spring and summer breeding. However, authors did not include population data from the Midwest, which is a more important area for summer breeding (Flockhart et al. 2017) that has probably experienced greater habitat loss (Brower et al. 2012, Pleasants and Oberhauser 2013). Badgett and Davis (2015) proposed that more conservation efforts should be directed to support successful migration. That would include protecting habitats along the migratory flyway and securing nectar sources as well as roosting sites. Roadkill and other man-made sources of mortality should be investigated, especially in areas where monarchs become concentrated, such as in Texas. They also advise limiting the spread of non-native *A. curassavica* to maintain the natural migratory behavior of monarchs.

Inamine et al. (2016) analyzed the relationship between butterfly population indices throughout the migratory cycle and concluded that events during the fall migration may be more critical to the monarch decline than milkweed limitation. They also studied seasonal trends in NABA data during a 22-year period, and found that northern monarch populations are able to build up during the breeding generations, despite the acknowledged decline in the overwintering sites. They found that the decline in the first generation in the southern USA can be fully accounted for by the decline in the overwintering population. According to their results, the population decline is driven by events after reproduction on milkweed during the spring and summer. Furthermore, they suggest that an unknown, annually increasing effect is affecting monarchs by the time they reach overwintering sites causing consistent decline in the population density. They suggest that factors reducing fall migration success should be further investigated, such as sub-lethal impacts of poor quality summer breeding habitat, including insecticide residues, lack of nectar resources, habitat fragmentation, and degradation of suitable overwintering sites.

3.4.2. Road Mortality

Road mortality has been identified as a threat to migrating monarchs in Illinois, where an estimated 500,000 monarchs were killed statewide during one week in August through October of 1998 (McKenna *et al.* 2001). Badgett and Davis (2015) suggested investigating monarch roadkill in Texas and northern Mexico as a source of fall migration mortality. Our Texas Central Flyway monarch roadkill survey data from the fall of 2016 found heaviest monarch roadkill along Interstate 10 between Sonora and Sheffield during October, apparently associated with large roosts in trees at a rest stop in the semi-arid region. We used this survey data to develop a fall migration roadkill maxent niche model, with which we estimated from 230,000 to 1.1 million monarchs were killed in 2016 from Texas to Mexico along the Central Flyway (Coulson et al. unpublished).

Along the Central Flyway in all three of the northern states of Mexico, including Tamaulipas, Nuevo Leon and Coahuila, monarchs have been observed flying low over roads, and roadway speed reductions have been implemented to protect monarchs in at least two states. According to an official from the National Commission of Protected Natural Areas (CONANP), signs were posted in Coahuila state in October 2015 limiting the maximum speed to 60 km per hour (37



mph) for roads inside the monarch migratory route to reduce monarch road mortality, especially along Federal Highway 57, which runs from Piedras Negras (Eagle Pass) to Saltillo (Miranda 2015). Police have been observed slowing traffic in Nuevo Leon state to reduce mortality of monarchs crossing a highway south of Monterrey, and monarchs have been observed flying low out of a ravine over a road southeast of Ciudad Victoria in Tamaulipas state (Chip Taylor, personal communication). In Taiwan, migrating milkweed butterflies related to monarchs, purple crow butterflies (*Euploea* spp.), are protected during their migrations crossing National Freeway No. 3, where they have experienced high roadkill mortality. The protections consist of the placement of a four-meter-high net for a length of 400 meters to induce the butterflies to fly over and above the traffic, and the closing of traffic in the outer lane when more than 500 butterflies per minute are passing (Her 2008).

3.4.3. Milkweed and Nectar Sources

Ensuring nectar sources along the migration routes is important (Brower et al. 2006). Conservation efforts at breeding or overwintering habitats are not effective if monarchs do not survive the long journeys between them (Baker et al. 2004, Brower et al. 2006). These ‘fueling’ areas has to be protected from threats, such as habitat destruction, invasive species, and introduced predators

Literature Review

(Brower et al. 2006). According to Cane and Tepedino (2001), distribution, phenology, and abundance of nectar sources will vary, both inter-annually with weather conditions, and over a longer time period, as a result of climate change, ecological interactions with exotic species, shifting agricultural practices, and other land use changes.

Nabhan (2004) suggested that migratory species are particularly vulnerable to the lack of nectar sources, as they may be dependant upon very localized nectar corridors along their migration routes. During migration, monarchs should not be sensitive to changes in vegetation within small areas or loss of an individual plant species (Brower et al. 2006). Landscape level changes will probably affect the ability to store lipids to survive the winter (Brower et al. 2006). However, sufficient data to test this hypothesis does not so far exist (Brower et al. 2006).

3.4.4. Insecticides

Fall migrating monarchs often roost in pecan trees in west Texas (Journey North 2017b), where they have also been observed to feed on the honeydew exuded by pecan aphids around San Angelo (C. Allen, personal communication). Pecan orchards are sometimes treated with systemic neonicotinoid insecticides (Imidacloprid) for black pecan aphids (Knutson and Rees 2002) as late as the end of September (C. Allen, personal communication; A. Knutson, Texas AgriLife Extension, Dallas, Texas, personal communication). The potential for migrating monarchs to be exposed to imidacloprid from feeding on pecan aphid honeydew during October in west Texas requires further consideration.

3.5. Winter Breeding

As previously discussed, overwintering roosts of monarchs have not been reported from Texas (Howard et al. 2010). However, breeding adults and larvae are known to occur along coastal and south central Texas where they are associated with cultivated *A. curassavica* in gardens (Prysby and Oberhauser 2004, Howard et al. 2010, Batalden and Oberhauser 2015).

3.6. Natural Enemies

3.6.1. Impacts of natural enemies

Herbivorous insect populations, in general, are affected by natural enemies (Kollberg et al. 2014). Various predators and parasitoids affect monarch butterflies during the life- and annual cycles, especially at egg, larval, and pupal stages (Oberhauser et al. 2015). Interactions between prey and the natural enemies are complex and difficult to predict and interpret (Turchin et al. 2003, Speight et al. 2008). Impacts of natural enemies on a species population depends on various factors, such as life cycle and population density of the host species, habitat characteristics, prevailing climatic conditions, and season of year (Kollberg et al. 2014, Netherer and Schopf 2010, De Anda and

Oberhauser 2015).

Natural enemies are likely to have more significant impacts on immature monarchs than the adults. Scientists have reported an overall survival rate from egg to late-instar larval stage of less than 10% (e.g., Prysby 2004, Calvert 2004, De Anda and Oberhauser 2015, Nail et al. 2015). Besides of natural enemies, potential mortality factors of immature monarchs include resource availability, temperature, and insecticides. Due to the high mortality of eggs and larvae, immature mortality may be an important driver of monarch populations (Nail et al. 2015).

Monarchs' interaction with milkweed hosts further affect their interactions with natural enemies (Oberhauser et al. 2015). Cardenolides, also found in milkweed, are a diverse group of steroids typically used in plant defence (Agrawal et al. 2012). Monarchs store cardenolides from the milkweed host during the larval stage (Malcolm and Brower 1989). Immediate levels of cardenolides have been found in most eggs (Zalucki et al. 1989). Both larvae and adult monarchs can sequester these compounds at high levels for their own defence (Malcolm and Brower 1989, Malcolm 1995). Toxicity by cardenolides combined with aposematism appears to provide some level of protection from predation for immature monarchs (Reichstein et al. 1968). However, the degree of protection afforded by these compounds is poorly understood (Oberhauser et al. 2015). Monarchs have many natural enemies that can either tolerate or avoid their sequestered cardenolide toxins (Oberhauser et al. 2015).

3.6.2. Predation

At the overwintering sites in Mexico, predation of at least 9% by birds and 5% by mice has been observed (Brower and Calvert 1985, Brower et al. 1985, Glendinning et al. 1988). The only invertebrate species observed to predate on adult overwintering monarchs so far are wasps (Leong et al. 1990). Observations of adult monarch predators during the breeding season include birds (McIsaac 1991) and invertebrates, such as spiders, mantids, and dragonflies (Smithers 1973, White and Sexton 1989).

(Prysby 2004) included ants, spiders, and red velvet mites as potential predators of immature monarchs at a field site in Wisconsin. De Anda and Oberhauser (2015) investigated stage specific mortality of monarch eggs and larvae on *A. syriaca* in Minnesota. They observed predation events by lacewing larvae, stink bug nymphs, and spiders. However, only the predation by spiders correlated significantly with monarch survival. other potentials predators present, included ants, syrphid fly larvae, and Asian lady beetles.

Calvert (1996, 1999) observed from egg to 3rd instar larval predation by red imported fire ant in Texas and proposed these ants are a significant predator of monarchs in the area. Lynch and Martin (1993) frequently observed monarch larvae predation by crab spiders and ants in Texas and Louisiana. Smaller larvae are more susceptible to predation and other stressors than later staged larvae (Altizer et al. 2000, Kingsolver and Huey 2008). However, Zalucki and Brower (1993) suggested that host plant characteristics are affecting monarch 2nd instar larval survival more than

natural enemies. For example, 1st instar larvae can have reduced survival due to getting mired in sticky latex or from cardenolide poisoning. Damaged milkweed leaves exude milky latex as a plant defense, which is usually also rich with cardenolides (Zalucki and Brower 1992, Zalucki and Malcolm 1999). Oberhauser et al. (2015) investigated pupal predation and parasitism. They documented prepupae and pupae predation by paper wasps (*Polistes dominula*) for the first time. Larval predation by paper wasps had been recorded previously (Rayor 2004).

3.6.3. Parasites and Parasitoids

OE is the most studied monarch parasite (Altizer and de Roode 2015). OE is host specific to monarchs, with a related strain that parasitizes queen butterflies (Altizer et al. 2000, Bradley and Altizer 2005). Adult monarchs infected with OE emerge from the chrysalises covered with thousands of dormant spores (McLaughlin and Myers 1970). Infected adults disperse parasite spores onto eggs and milkweed, and after, larvae ingest spores while feeding (Altizer et al. 2004). OE does not transmit from larva to larva. OE can also be transmitted from adult to adult during mating or other contact (Altizer 1998). OE infection increase adult monarch mortality, especially in dry conditions (Altizer and Oberhauser 1999). Heavily infected monarchs suffer from wing deformities, reduced body size, flight performance, and lifespan (Altizer and Oberhauser 1999, Bradley and Altizer 2005). OE infections occur in all monarch populations, but populations that migrate longer distances have lower infection prevalence (Altizer et al. 2000, Altizer and de Roode 2015).

Parasitoids feed in or on a host species eventually causing death of an organism (Oberhauser et al. 2015). Monarch parasitoids includes tachinid flies and at least a species of brachonid wasp (Arnaud 1978). Oberhauser et al. (2015) observed pupal parasitism by a parasitoid *Pteromalus sp.* wasp. Tachinid flies attack monarch larvae of all instars (Oberhauser 2012), but mortality does not occur until the 5th instar to adult stages (Oberhauser 2012, Nail et al. 2015) In general, monarch mortality by parasitoids typically occurs after pupation (Nail et al. 2015). In several studies, parasitism rates by tachinid flies have varied between 1% and 100% (reviewed by Oberhauser et al. 2015). Years with high monarch densities tend to be followed by years with higher tachinid fly parasitism rates (Oberhauser 2012). In Texas, 43% parasitism of monarchs by tachinid flies has been observed (Lynch and Martin 1993).

4. Conservation Strategies

4.1. Overview

The North American Climate, Clean Energy, and Environment Partnership Action Plan (Trudeau, et al. 2016) has identified a monarch restoration target of six hectares of Mexican overwintering sites occupied by monarchs by 2020. According to Thogmartin et al. (2017), this equals approximately 127 million monarchs. Subsequently, Thogmartin et al. (2017) estimated that 3.62

billion stems of milkweed is required to support this population of 127 million. A study by Pleasants (2017) suggested that additional 1.6 billion milkweed stems is needed to support the goal of six hectares of overwintering monarchs.

Monarch production in the Midwest is higher in milkweed growing in crop fields than in non-agricultural areas (Pleasants 2017). In a study by Pleasants and Oberhauser (2013), milkweeds in corn and soybean fields had 3.9 times more eggs per stem compared to non-croplands. This ratio needs to be taken into account in milkweed restoration efforts (Pleasants 2017).

Flockhart et al. (2017) conclude that conservation across the entire larval development range of the monarch is important to buffer against regional climate variability. The importance of rangewide conservation is also supported by the population model of Oberhauser et al. (2017). They found that monarch habitat in the south and north central parts of the breeding range may be especially important for restoration that effectively increases monarch population growth. Mueller and Baum (2014) summarize two overall goals for conservation strategies: (1) reducing habitat fragmentation and loss of overwintering habitats in Mexico; and (2) increasing nectar and host plants throughout the breeding range. Mueller and Baum (2014) point out that roadsides may provide important habitat for monarchs, and suggest that monarch habitat needs should be taken into account when roadside management strategies are developed.

4.2. Grasslands

4.2.1. Milkweed and Nectar Plant Conservation

The USDA NRCS (2016), together with US Fish and Wildlife Service, have developed the Southern Great Plains (South Central) Habitat Strategy for enhancing monarch habitat in Kansas, Oklahoma, and Texas. The plan prioritizes incentivization for the conservation and protection of existing milkweed stands in the southern Great Plains. Their main strategy involves promotion of converting invasive monoculture grasslands to species rich native grasslands and the implementation of grazing systems that maximize species richness, including nectar species. Various management practices were identified with the potential to increase milkweed and nectar plant populations in grasslands, including brush management, prescribed burning, prescribed grazing, and mowing.

Late season sustained mowing of grasslands in Oklahoma (in June and September, or September) increased the cover of broadleaf species such as *A. viridis*, while suppressing invasive KR bluestem (Dee et al. 2016). Encouraging prescribed grazing, burning, or mowing of milkweed patches in grasslands and pastures at the proper time of summer, can also yield fall milkweed resprouts that can increase the production of fifth generation migrating adults (Baum and Sharber 2012, Baum and Mueller 2015). Alcock et al. (2016) and Fischer et al. (2015) report that mowing fields or hay pastures during the early summer can also promote late season resprouts of *A. syriaca* that are used by monarch larvae.

4.2.2. Milkweed Plantings

Various organizations (e.g., The Xerces Society [Borders and Lee-Mäder 2014], Monarch Watch 2017c, Monarch Joint Venture [MJV] 2017) additionally promote the propagation and planting of milkweeds in grassland restoration, including the southern plains region in Texas and Oklahoma. Milkweeds are generally planted as seeds or transplants in areas where the existing vegetation has been cleared, such as through shallow cultivation and repeated applications of herbicide (Luna and Karsten Dumroese 2013; Borders and Lee-Mäder 2014, 2015). Nail et al. (2015) found a positive relationship between immature monarch survival and number of monitored milkweed plants. They proposed planting of milkweed in large numbers as a conservation effort. Not just to support monarch populations, but to possibly support higher survival rate.

4.3. Rights-of-Way

Strategies have been developed for conservation and creation of monarch habitat for milkweeds and nectar plants in roadsides, railways, and utility rights-of-way (Harper-Lore and Wilson 2000, Rager et al. 2013, MJV 2015, 2017). Strategies for general pollinator conservation on roadways include (1) conservation and planting of regional native nectar plants, (2) adjusting of mowing schedules to benefit desired plants, and (3) reduced use of herbicides (Hopwood et al. 2015, Federal Highway Administration 2017, MJV 2017). The Texas Department of Transportation (TxDOT) Wildflower Program that includes delayed mowing until spring and fall flowers have gone to seed (e.g., MJV 2015), and seeding of roadsides with the aid of a regionally specific native plant list. These TxDOT activities benefit both monarch nectar plants and milkweeds, and they have also partnered with USDI FWS and the Native Plant Society of Texas to establish at least four monarch gardens of milkweeds and nectar plants at safety rest stops, including along Interstate Highway 35 (Texas Parks and Wildlife Department [TPWD] 2016).

4.4. Urban Areas

The National Wildlife Foundation has developed best management practices for monarch butterfly conservation in urban areas, including planting of monarch gardens with nectar plants and milkweeds. They have enlisted the participation of many cities, including a number in Texas (Fitzgerald 2015). Butterfly or pollinator friendly gardens and using native plant species in landscaping may enhance monarch habitats locally. However, they will never compensate for the loss of native nectar sources within rural habitats (Brower et al. 2006).

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APPENDIX A:
MANUSCRIPT SECTION 1;
MODELING FALL MIGRATION PATHWAYS AND SPATIALLY
IDENTIFYING POTENTIAL MIGRATORY HAZARDS FOR THE EASTERN
MONARCH BUTTERFLY

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Abstract

Context Identifying core migratory pathways and associated threats is important for developing conservation priorities for declining migratory species, such as eastern monarch butterflies (*Danaus plexippus*).

Objectives Characterization of monarch fall migration core pathways and annual variability was compared using kernel density estimation models (KDEMs) and MaxEnt ecological niche models. Potential anthropogenic hazards were identified across migratory pathways and related to conservation strategies.

Methods Journey North citizen scientist monarch overnight roost data from 2002–2016 were used to model the fall migration at 10 km spatial resolution with MaxEnt and KDEMs. Potential anthropogenic threats to the fall migration were spatially identified along the core migration routes.

Results The KDEM migratory pathways best represented patterns of monarch movement towards overwintering locations. Migratory routes varied as much as 200 km from east to west in the southern Central Flyway, which was also the only area identified with monarch roadkill hotspots. Potential threats from mosquito adulticide ultra-low volume (ULV) spraying were concentrated along Eastern Flyway coastal areas. Potential nectar resource loss or contamination from high usage of glyphosate herbicide and neonicotinoid insecticides was greatest in the Midwest, within the core route of the Central Flyway.

Conclusions MaxEnt and KDEM were complementary in modeling monarch migratory pathways. Monarch roadkill estimation and mitigation strategies are most needed in the southern core migratory pathways through Texas and Mexico. Enhancement of nectar resources to mitigate for potential threats from mosquito ULV spraying and nectar resource loss or contamination are most critical for coastal areas and the Midwest, respectively.

Keywords Citizen science, niche models, KDE models, migratory threats, mitigation conservation, pollinators.

Introduction

Many migratory species of conservation concern are declining worldwide (Hardesty-Moore 2018). Comprehensive conservation strategies for these species must take into account breeding, migratory stopover, and overwintering sites. Annual mortality for migrant species, especially birds, can be highest across migratory routes, particularly in narrower portions of migratory pathways. Spatial models of migratory pathways are an important tool for identifying conservation priorities (Runge et al. 2014). Common approaches for defining migratory pathways include kernel density estimation (KDE), and correlative ecological niche models, such as MaxEnt. These methods have not been thoroughly compared to evaluate their contributions to migratory pathway analyses.

Kernel density estimation is a nonparametric spatial point pattern method for analyzing point intensity that is often used with telemetry data for assessing the frequency of utilization of an area in defining animal home ranges (Worton 1989; Kie et al. 2010). The KDE intensity surface has also been used to delineate migratory movement, particularly for birds, based on telemetry data of stopover locations for certain seasons (Rayner et al. 2017), or over the entire migratory pathway (Pierce et al. 2017; Wytinski and Bonter 2018). Moore and Krementz (2017) used non-telemetry bird band return data with KDE to analyze migration origin and destination locations. We are not aware of the previous use of KDEs in defining the entire migratory pathway with non-telemetry data as done in this study. Correlative ecological niche models, particularly MaxEnt, have been

used to model migratory pathways using the same types of telemetry data in conjunction with environmental layers (e.g., Huff et al. 2012; Williams et al. 2017). Migratory niche models have also been incorporated into a larger framework for delineating migration routes, either as resistance layers in connectivity models for ungulates (Bond et al. 2017), or as a refueling resource layer in individual-based models for birds (Smith and Deppe 2008).

The KDE intensity surface has also been used in developing interpolative KDE models (KDEMs) of species distributions (Pennay et al. 2011). We develop novel training set ensembles of migratory KDEMs from normalized KDE surfaces, performing calibration and testing in the same manner as niche models. The KDEMs facilitate more detailed analyses of migration pathways, including comparisons across years and with MaxEnt models. Interpolative KDEMs are restricted to projecting occurrence over an area limited by the radius of the selected KDE bandwidth around the training data points. In contrast, correlative niche models incorporate environmental data to create a predictive surface that can be extrapolated beyond the boundaries of the training data. While migratory KDEMs cannot directly incorporate environmental layers, they should be more sensitive to clustered point patterns related to organismal movement (endogenous autocorrelation) that have little or no relationship to the spatial distribution of environmental conditions (exogenous correlation) (c.f., Bahn and McGill 2007). We evaluate the use of KDEMs and MaxEnt in analyzing migratory pathways for the eastern monarch butterfly (*Danaus plexippus*) (Nymphalidae: Danainae).

The migratory phenomenon involving the same adult eastern monarchs traveling from as far north as southern Canada to Central Mexico in the fall and back to the southern US in the spring is in danger of disappearing (Brower et al. 2012). The migrant overwintering population has potentially declined as much as 80% over the past two decades (Vidal and Rendón-Salinas 2014). Many studies have focused on habitat loss within the breeding grounds (Brower et al. 2012; Pleasants 2017; Malcom 2018; Stenoien et al. 2018) and at overwintering sites (Brower et al. 2012; Vidal et al. 2014; Malcolm 2018). Thogmartin et al. (2017) modeled the impact of various factors on monarch population decline, including climate, disease, and agricultural insecticide use (neonicotinoids), and observed strong negative effects from all of these, particularly glyphosate herbicide use. Mortality during the fall migration has also been suggested as a significant factor in the monarch decline (Badgett and Davis 2015; Ries et al. 2015; Inamine et al. 2016; Agrawal and Inamine 2018). However, fewer studies have focused on potential anthropogenic threats to the fall migration (but see McKenna et al. 2001).

Two main flyway routes have been described for the fall migration of the eastern monarch based on overnight roost sites: (1) the Central Flyway, bounded to the east by the Appalachian Mountains and to the west by the Rocky Mountains; and (2) the Eastern or Coastal Flyway, following the Atlantic and Gulf coasts (Calvert and Wagner 1999; Howard and Davis 2009). Overnight roosting behavior, where few to several thousand monarchs aggregate within trees and shrubs, is almost exclusively associated with the fall migration (Davis and Garland 2004; Brower et al. 2006; Howard and Davis 2009). Journey North (2017) maintains an extensive citizen science database of monarch sightings since 2002 that includes overnight roost locations. Models of the migratory pathways developed from these data can provide important information on yearly variation in migration routes, with implications for spatially assessing conservation priorities and threats.

A variety of potential anthropogenic hazards to adult fall migrating monarchs have been proposed (Agrawal and Inamine 2018; Malcom 2018). We focus on four relatively important potential threats identified in the literature. Two threats have been previously reported as mortality

agents for fall adult migrating monarchs, roadkill (McKenna et al. 2001; Tracy 2018; see also Baxter-Gilbert et al. [2015]) and ultra-low volume (ULV) spraying of insecticides (e.g., resmethrin and permethrin) for adult mosquito control (Journey North 2000; Oberhauser et al. 2006, 2009; see also Barger [2012]; Hoang and Rand [2015]). The two additional potential threats include reduced fitness from scarcer nectar resources due to increased glyphosate herbicide use on agricultural lands (Brower et al. 2006), and reduced fitness from exposure to agricultural pesticides, particularly systemic neonicotinoids contaminating flower nectar sources near agriculture (Stenoien et al. 2018). Potential sublethal effects of reduced fitness for any of these threats have not yet been demonstrated (Braak et al. 2018; Stenoien et al. 2018). Spatial identification of potential threats is needed along the fall migratory routes, especially along core pathways approaching the overwintering sites (Brower et al. 2006; Badgett and Davis 2015), and it is prerequisite to further migratory risk assessments (e.g., Liechti et al. 2013).

The goals of this study are to compare KDEM and MaxEnt for defining the core fall migration pathways of the eastern monarch, and to identify the above listed potential migratory hazards along these pathways. The specific objectives are to (1) project and compare the eastern monarch fall migratory pathways with the two approaches of MaxEnt and KDEM; (2) examine annual variability in the migration pathways, delineating core migration routes; and (3) spatially identify potential hazards to the fall migration, analyzing implications for monarch conservation planning and future migratory risk assessment.

Methods

Study site

We followed Brower (1995) in regarding the Rocky Mountains and Sierra Madre Oriental as the western boundary for the eastern monarch (Fig. 1). The southern boundary in Florida follows the boundary of the spring breeding range as delimited by Oberhauser et al. (2017). The background evaluation extent for training and evaluation of monarch migration models is derived from a 500 km buffer around a convex hull polygon formed using the 2002 to 2016 overnight roost records from Journey North (2017) (Fig. 1). This background evaluation extent encompasses most of the range of the eastern monarch migratory population.

Overnight roosting records

Journey North citizen science volunteers have been reporting their spring monarch observations to the program since 1997, and records of overnight roosts since 2002 (Journey North 2017). We compared geocoordinates with the location descriptions in the comments of roosting records and determined that the spatial accuracy of the data was approximately ± 5 km. Consequently, we aggregated the roosting locations across a raster with spatial resolution of 10 km^2 . A total of 2,803 roosting records from the 15 years of 2002–2016 were used in the study, ranging from 25 (2002) to 422 (2015) records per year (Table B.1). We regarded all Journey North roost records within a 10 km^2 area as indicative of the presence of migratory roosting individuals within a given year, regardless of butterfly count data per roost.

MaxEnt migration pathway models

Niche modeling

An initial set of 80 environmental variables generalized to 10 km resolution were used in developing the MaxEnt niche models, including 57 climatic indices, 12 topographic indices, and 11 land cover indices (Table B.2). MaxEnt version 3.3.3 models (Phillips et al. 2006) were developed from monarch roost locations using the R-software (R Core Team 2017) *dismo* package (Hijmans et al. 2011). The aggregation of roost locations to 10 km served as a 10 km spatial filter for reducing sample bias and spatial autocorrelation among the data (Boria et al. 2014). About 10,000 pseudoabsence points were generated for model evaluation within the background evaluation extent. The pseudoabsences were buffered at 20 km from the presence points (e.g., Barbet-Massin et al. 2012). In order to standardize the performance statistics used in comparing MaxEnt with KDEM models, the R PresenceAbsence package (Freeman and Moisen 2008) was used with presence and pseudoabsence points for calculating a pseudoabsence (psa) version of the true skill statistic (TSS_{psa}) and area under the curve statistic (AUC_{psa}) using random cross validation. This is in contrast to the standard practice of utilizing the MaxEnt software default calculation of a background presence (bgp) version of AUC (AUC_{bgp}), which incorporates as absences both background and presence points (not buffered pseudoabsence points). To reduce MaxEnt model complexity and overfitting for improved model generalization (Jiménez-Valverde et al. 2008; Warren and Seifert 2011), we adjusted the MaxEnt beta regularization to two and used only quadratic and hinge features (Tracy et al. 2018). The top 12 feature-selected MaxEnt models were calibrated to binary presence/absence format using a threshold of maximum TSS_{psa} (Liu et al. 2013). These models were combined using frequency consensus to form an ensemble of models derived from different selected feature subsets (feature subset ensemble). The AICc was calculated for the 12 models from the MaxEnt model raw version rasters and training presence point data using the ENMeval R package (Muscarella et al. 2014).

Feature selection

We employed a random subset feature selection algorithm (RSFSA; Tracy et al. 2018) to select an ensemble of estimated optimally sized subsets of 80 environmental variables or predictors (environmental features comprising feature subsets; Table B.2). The objective of RSFSA was to produce MaxEnt models exhibiting higher accuracy (AUC_{psa}), lower complexity, as measured by lower corrected Akaike information criterion (AICc), and lower overfitting, as measured by AUC_{psa_diff} (training AUC_{psa} minus test AUC_{psa} ; Warren and Seifert 2011) than random feature subsets. The RSFSA utilizes two types of search criteria in selecting feature subset ensembles producing higher performance MaxEnt models: (1) a correlation threshold of $|0.7|$ (Dormann et al. 2013) for inclusion of variables in thousands of randomly generated feature subsets, and (2) the AUC or AICc statistic (whichever performs best) to rank the thousands of random feature subset derived MaxEnt models. In each of two RSFSA stages, held out model training and testing data is used to evaluate the performance of top ranked feature subset MaxEnt models compared to random feature subset MaxEnt models. In stage I, several hundred MaxEnt models are developed for each of a range of feature subset sizes, and an optimal subset size is selected beyond which gains in model performance are negligible. In stage II, several thousand MaxEnt models are developed for the selected feature subset size, ranked by AUC or AICc, and their performance evaluated. A final set of 12 top ranked models is then selected to form a feature subset ensemble. We jointly ranked the variables in the top 12 feature-selected MaxEnt models for mean variable permutation

importance (e.g., Halvorsen 2013) and frequency of variable appearance in the models (for details, see Supplementary material, Methods, and Tracy et al. 2018).

Kernel density estimation model migration pathways

The KDEs were developed at 10 km resolution using presence-only monarch roost locations with the ArcGIS (ESRI Inc., Redlands, California) Kernel Density tool (Geodesic method, points as values), which employs a quartic (biweight) kernel function. We used the North America Albers Equal Area Conic Projection (North American 1983 datum) as the required equal area projection for KDE. The optimal KDE bandwidth (smoothing parameter) was automatically calculated in ArcGIS by a spatial variant of Silverman's Rule-of-Thumb that is robust to outliers. To compensate for higher numbers of observers in densely populated areas, monarch overnight roost locations at 10 km resolution were weighted by a grid of mean human population density per 1 km for the year 2000 (Center for International Earth Science Information Network, CIESIN, 2005) (for details, see Supplementary material, Methods). The KDE raster was then calculated and normalized from zero to one to create a raw KDEM surface of probability of occurrence. The final KDEM was created by restricting the boundaries of the raw KDEM to that of a calibrated binary presence/absence version using a threshold of maximum TSS_{psa} as was done for MaxEnt models. A KDEM without any adjustment for human population was also calculated for comparison.

The raw KDEM probability surface was used for calculation of AICc (e.g., Graham et al. 2013) with the ENMeval R package. Values of zero were first changed to 0.000001 and all values recalculated to sum to one within the background evaluation area (analogous to the MaxEnt raw values used with ENMeval R). This recalculated raw KDEM raster and associated model values for training presence points were then used in calculating AICc. Accuracy statistics of TSS_{psa} and AUC_{psa} were calculated for raw KDEMs in the same way as described above for MaxEnt. We calculated a total of three different KDEMs derived from standard random three-fold partitions of two-thirds training and one-third testing data using the 2002–2016 roost data. From the three training KDEMs, we developed a training set ensemble (TSE). From the TSE, a minimum consensus TSE model was derived representing the area encompassing at least one of the three training KDEMs. The minimum consensus TSE was used as a boundary for display of the average consensus of the continuous raster training KDEMs. The 100% consensus KDEM TSE boundary was also calculated for display (see Table B.4 for shapefile; R code and Arc python code for generating and analyzing KDEMs is available at <https://github.com/jamesltracy/KDEMs>). Both combined year and annual (see below) KDEMs were used in delineating monarch fall migratory flyways and core migration pathways, respectively (see Supplementary material, Methods, Flyways and core migratory pathways).

Comparison of MaxEnt and kernel density estimation model migration pathways

Statistical comparisons of AUC_{psa} , AICc and AUC_{psa_diff} (overfitting) between the 12 MaxEnt model feature subset ensemble and the three KDEM training set ensemble were performed using an R core implementation of the Welch t-test, which allowed for the case of unequal variance. Models were also evaluated for ability to identify different regions of the migration pathways as a basis for further analyses described below.

Annual kernel density estimation model migration pathways

We followed the same procedure described above to create three binary calibrated minimum frequency consensus training set ensembles of KDEMs for the annual fall migration routes for individual years from 2005 to 2016. These years were chosen based on a minimum requirement of 100 roosting records per year. Data were spatially thinned separately by year for annual models. These annual KDEMs were combined to form a 100% consensus 2005-2016 annual KDEM ensemble to highlight core migratory pathways. We calculated the means and standard deviations for the width and relative centroid shifts of annual KDEM migratory pathways for the northern (37-50°N latitude) and southern (27-37°N latitude) portions of the Central Flyway. The 37°N north-south boundary was based upon the approximate latitude where the migration pathway starts to narrow as it proceeds south along the Central Flyway. Low numbers of observations of annual roosts were not conducive to similar analyses for either the Eastern Flyway or areas south of 27°N latitude. Migration pathway width and centroid shift calculations were performed for the annual models and for the combined 2002-2016 model. Spearman correlation coefficients were calculated between annual average KDEM widths and centroid shifts, and the area of overwintering colonies in Mexico (Vidal and Rendón-Salinas 2014; Monarch Watch 2017).

Anthropogenic fall migration hazards

We identified reports of localized high monarch roadkill (roadkill hotspots) in eastern North America from citizen science data (including Correo Real, a Mexican monarch citizen science organization) and survey data, summarizing available data on roadkill rates per 100 m per single roadway edge. Locations and dates for mortality of monarch adults from mosquito ultra-low volume (ULV) adulticide spraying during the fall migration period were screened from internet news reports and assembled as an additional citizen science data source. The latest months for ULV mosquito spraying across representative cities and counties of the eastern US were screened from news and governmental internet sources, and these were compared with approximate monarch peak migration dates by latitude. We also quantified the area of US counties by states for various monarch flyway regions (Fig. B.3) with potential mosquito ULV spraying also indicated from screened governmental and news internet sources (for sources, see Results and Supplementary material). The US counties with 75th percentile usage of both glyphosate and combined major neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) during 2014 (EPest-high method, kg/harvested crop acre; US Geological Survey [USGS] 2018) were also quantified by area for various flyway regions. These areas should reveal the greatest potential threats of intensified agricultural development to fitness of migrating adult monarchs from reduced nectar resources and insecticide exposure.

Results

MaxEnt migration pathways

The random subset feature selection algorithm identified 12 six-variable MaxEnt models with higher AUC_{psa} values than random models, ranging from 0.968 to 0.982 (Fig. B.1; for details, see Supplementary material, Results). A total of 42 out of 80 variables were used at least once in the 12 selected models (Tables B.2, B.3). The ten variables jointly ranked highest by MaxEnt model permutation importance and frequency of appearance in the 12 models were all climatic indices

(Table B.3). Four of these top ten climate variables were Supplementary Climatic indices, four were Actual and Potential Evapotranspiration (AET-PET) indices, and two were Bioclim indices. The top three ranked variables were the Supplementary Climatic index of spring monthly minimum temperature, and two AET-PET indices of autumn mean monthly actual evapotranspiration, and total annual actual evapotranspiration. The 11 land cover indices generally had the lowest MaxEnt permutation importance and frequency of appearance in the 12 selected models (Tables B.2, B.3).

The 100% consensus of the 12 calibrated MaxEnt models is mostly concentrated within the Central Flyway (Fig. 2A). A large portion of the MaxEnt 100% consensus boundary for the Central Flyway coincides with the grasslands of the Great Plains Level I ecoregion (Commission for Environmental Cooperation, CEC, 2005) (Fig. 2A). The MaxEnt 100% consensus boundary includes only northern portions of the Eastern Flyway.

Kernel density estimation model migration pathways

The AUC_{psa} values of the three training KDEMs for the entire 15-year data set (2002–2016) ranged from 0.995 to 0.998 (default KDE bandwidths ranged from 226 to 231 km). The KDEM 100% consensus boundary is mostly associated with the Central Flyway, but it includes portions of the Eastern Flyway in the Northeast and coastal Alabama and the Florida panhandle (Fig. 2B). The Great Plains ecoregion overlaps much of the KDEM boundary in the Central Flyway (Fig. 2B). The eastern portion of the Chihuahuan Desert Level III ecoregion is also included in the KDEM boundary. In addition, the KDEM boundary includes several Level I ecoregions that approach or encompass the overwintering sites in central Mexico. Much of the KDEM boundary for the Eastern Flyway is occupied by Level II coastal plains ecoregions (Fig. 2B). A lower projected density of roosting is apparent in forested Level II ecoregions of the Eastern Flyway (see Supplementary material, Results, for models with and without adjustment for observer bias using human population density).

Comparison of MaxEnt and kernel density estimation model migration pathways

Accuracy (AUC_{psa}) of the KDEM training set ensemble was significantly higher than that of the MaxEnt feature subset ensemble in projecting the monarch migration pathways (Fig. 3A). However, model complexity (AICc) was significantly lower in the MaxEnt ensemble (Fig. 3B). We found no significant difference in model overfitting between MaxEnt and KDEM ensembles (Fig. 3C). The KDEM consensus boundary better distinguished the migratory pathway through the western Chihuahuan Desert and Mexico where the MaxEnt consensus boundary was mostly absent (Fig. 2). In addition, the KDEM boundary incorporated larger portions of the Eastern Flyway. Consequently, the 2002-2016 consensus KDEM was used in updating boundaries of the Central and Eastern Flyway divisions (Figs. 1, S2-3, for details, see Supplementary material, Results, Flyways and core migration pathways).

Annual kernel density estimation model migration pathways

The migration pathways of annual KDEM training set consensus models varied considerably from year to year between 2005 and 2016 in the Central Flyway division (Fig. 4B-M). The greatest observed annual centroid shift measured from the 2002-2016 KDEM centroid was an

approximately 200 km westwards shift in the southern migratory pathway for 2015 (Figs. 4L, B.4F). With a single exception, no significant relationships were found between dimensional characteristics of the annual KDEMs and the areas of overwintering monarchs in Mexico for the same year (Fig. B.5). The exception was a significant, but weak, correlation ($r_s = 0.48$, $R^2 = 0.21$; $p = 0.0031$) indicating the association of a western shift in the north centroid of the Central Flyway KDEM (Fig. 1) and a lower area of overwintering monarchs in Mexico (Fig. B.5C) (for further details, see Supplementary material, Results). The 2005-2016 consensus annual KDEM was used to reveal the core migratory pathway in the Central Flyway and define the southern Central and Coastal funnel core pathways in the southern portions of the flyways (Figs. 5, B.2-3, B.6; for details, see Supplementary material, Results, Flyways and core migration pathways).

Anthropogenic fall migration hazards

Seven reports of monarch October/November roadkill hotspots were identified, four along the Central Funnel in Texas (Journey North 2017, Tracy 2018) and three in Mexico (Correo Real 2015) (Table B.5; Figs. 5A, B.6A). In West Texas, two hotspots had 52 and 66 roadkill monarchs per 100 meter along Interstate Highway 10 west of Sonora and Ozona, respectively (Tracy 2018). In Mexico, two hotspots southwest of Monterrey had 250 and 575 roadkill monarchs per 100 m on Mexico Federal Highway 40D (Correo Real 2015). Several surveys along a 27 km stretch of Mexico Highway 40D yielded an average of 10 roadkill per 4 m (250/100 m) which represents 2,500 roadkill per km (Correo Real 2015).

Four reports of dozens to hundreds of dead adult fall migrating monarchs attributed to mosquito adulticide ULV truck spraying were found from 1999 to 2011. These were located in Central Park, New York (New York Daily News 1999; malathion), Gaylord, Minnesota (Journey North 2000; permethrin), Grand Forks, North Dakota (Grand Forks Herald 2010; with photo), and Santa Catarina, Monterrey, Nuevo Leon, Mexico (www.Info7.mx 2011; with video) (Fig. 5A; see Table B.6 for details on dates and numbers of monarch deaths reported). Lethal levels of permethrin were found in monarch cadavers from the Gaylord, Minnesota site (St. Cloud Times, 2000). The latest fall ULV application dates in 42 of 45 (93%) eastern US cities and counties potentially coincided with monarch peak migration periods (Fig. 5A, Table B.7). Counties with potential mosquito ULV treatments are concentrated along the coastal Eastern Flyway, including 42% and 21% of states within the southern Eastern Flyway and northern Eastern Flyway, respectively (Fig. B.7A). States with greatest percent areas of counties with potential ULV spraying during migration in the Eastern Flyway include New Jersey, Delaware, Massachusetts, South Carolina, southern Georgia, coastal Louisiana, and the Florida panhandle (Figs. 5A, B.7A; Table B.8). Large scale, late season mosquito ULV spray applications following hurricanes can occur in additional counties in the Eastern Flyway (e.g., North Carolina 2018). Potential mosquito ULV spray counties comprise about 38% of the portions of Louisiana and Texas within the Coastal Funnel (Fig. B.6B). In contrast, only 9-10% of the states with mosquito ULV spraying in the Central Flyway are comprised of potential mosquito ULV spray counties (Figs. 5A, B.7B, Table S8).

An overlap of the upper 75th percentile usage of glyphosate herbicide and neonicotinoid insecticides was found in US counties for 19 states. Over 30% of the area of these states within the northern Central Flyway had high glyphosate/neonicotinoid usage, including over 75% of Iowa and Illinois, and over 50% of Indiana, Nebraska and North Dakota (Figs. 5B, B.8A; Table B.8). In comparison, corresponding percentages of high glyphosate/neonicotinoid counties for the other

flyway regions are 11% for the southern Central Flyway (Fig. B.8B), 6% for the southern Eastern Flyway, and 1% for the northern Eastern Flyway (Fig. 5B, Table B.8).

Discussion

Comparison of MaxEnt and kernel density estimation models

Both spatially interpolative KDEMs and MaxEnt niche models can be complementary for analyzing different aspects of migratory pathways. Similar to Bahn and McGill (2007), we found that spatially interpolative models could perform with higher accuracy than niche models. Both the higher accuracy (AUC_{psa}) and complexity ($AICc$) of the KDEMs compared to MaxEnt models appears to be related to the sensitivity of the KDEMs to more spatially complex endogenous spatial patterns of migratory movement towards the overwintering sites (c.f., Bahn and McGill 2007; Figs. 2-3). The KDEMs are probably also more sensitive to spatial patterns of observer bias from local human population densities, the effects of which were not completely removed. However, the boundaries of the KDEM migration pathways appears to be less influenced by this observer bias (Fig. B.3A-B). Although the spatially interpolative KDEMs might be expected to overfit the training data more than MaxEnt, we did not find significant differences in model overfitting between KDEM and MaxEnt.

The KDEMs best identified the migration pathway through southern portions of the Eastern Flyway around the Florida panhandle and the southern portion of the Central Flyway in Mexico (Fig. 2). The 100% consensus KDEM projection of the monarch fall migration pathway in Mexico includes several ecoregions either mostly or entirely absent from the 100% consensus MaxEnt models, such as eastern portions of the Chihuahuan Desert and the Temperate Sierras near the overwintering sites (Fig. 2B). These ecoregions in the southern portion of the Central Flyway do not necessarily reflect favorable environmental conditions present over most of the flyway, contributing to their poor representation in the MaxEnt projections. The ability of KDEMs to detect the endogenous directed movement pattern of monarchs approaching the Mexican overwintering locations leads to better projection of the migration pathway over most of Mexico compared to modeling of environmental suitability by MaxEnt. The isolated portions of the Eastern Flyway identified by KDEM (but not MaxEnt) probably also represent environmental conditions very different from the more strongly represented Central Flyway. Scarcity of roosting data from Mexico and the Eastern Flyway may have also hampered the MaxEnt model performance in these regions. Consequently, we utilized the 100% consensus KDEMs for representing the general fall migration pathways for the eastern migratory population of the monarch butterfly (Figs. 1, 2B, S3B). In contrast, the MaxEnt migration models best emphasize the correlation of large portions of the Central Flyway with grassland ecoregions that may provide a more stable source of nectar for migrating monarchs than more forested ecoregions to the east. More relaxed KDEM calibration thresholds may have better revealed some of the Eastern Flyway pathways, but the utilized threshold of maximum TSS has been shown to perform well for presence only models (Liu et al. 2013). The migratory pathways appear to follow suitable habitat for certain areas, only partly supporting the suggestion by Dingle and Drake (2007) that migratory pathways should correlate with suitable habitat.

Annual kernel density estimation model migration pathways

We found significant inter-annual variation in the location and width of the migration pathway within the Central Flyway division as defined by the annual KDEMs (Fig. 4). Calvert and Wagner (1999) also observed variation in width and location of the migration pathway of the Central Flyway in Texas from 1993-1995. Inter-annual variation in migratory pathways has been observed in a variety of species, but a solid trend in species migratory pathways can usually be identified (Dingle and Drake 2007). We consider the KDEM annual consensus boundary (Figs. 5, B.3C) as the best representation of the narrower core migration pathway for most years through the Central Flyway division.

A west to east shift in the centroid of the annual migration pathway in the northern Central Flyway (Fig. 1), from central Iowa towards northwestern Illinois, was weakly but significantly associated with a larger area of overwintering roosts in Mexico (Fig. B.4C). Recent studies have indicated that the Upper Midwest from eastern Colorado to Indiana that encompasses the region of this shift represents the source for a large proportion of the overwintering population (Flockhart et al. 2017). A west to east shift in the densities of roosts for the northern migration pathway may indicate an increased source population of migrating monarchs to the east. Further investigation is needed to confirm the significance of the observed weak correlation between larger overwintering areas and an eastern shift in the northern migration pathway through the Central Flyway.

Anthropogenic fall migration hazards

We assembled additional evidence of monarch fall migration mortality from roadkill and ULV mosquito spraying in new areas of the migration pathways. McKenna et al. (2001) estimated that as many as 500,000 monarchs were killed in Illinois within the first week of September 1999. We report additional incidences of fall monarch roadkill hotspots in the Central Funnel region of Texas and Mexico. More detailed surveys are needed for estimating fall monarch road mortality across the Central and Coastal funnels.

Descriptions for four isolated instances of fall migrating monarch mortality related to mosquito ULV spraying, particularly in Monterrey, Mexico (www.Info7.mx 2011), indicate that spray fog contacted roadside trees in which monarchs were roosting overnight. Vegetation cover probably provides some protection from direct contact with spray droplets for roosting monarchs (c.f., Peterson et al. 2016), but typical evening and night ULV treatments to reduce non-target pollinator impacts (Ginsberg et al. 2017) can still expose roosting monarchs to lethal doses of mosquito adulticide treatments. Three of the four instances of monarch mosquito spray mortality were above 40°N, but the area for potential for mosquito ULV spraying during monarch peak migratory periods was mostly concentrated further south along the coast from New York to Texas, including the Florida panhandle. Although reported monarch roost mortality events from ULV mosquito spraying are localized, the lethal and sublethal impacts to monarchs from widespread ULV mosquito control along the coast of the Eastern Flyway during peak adult migration, especially from aerial naled applications (c.f., Barger 2012; Hoang and Rand 2015), warrants further investigation.

Widespread high usage of glyphosate and neonicotinoids occurs in Midwest agricultural areas of the monarch core migration pathways within the Central Flyway. The effects to adult monarch fitness and migratory success from potential reduction or pesticide contamination of nectar resources are not known. The potential influence of these threats on migratory connectivity could be investigated through determining differential recovery rates at Mexican overwintering sites for monarchs tagged at different sites along the migratory pathway in combination with

measurements of monarch lipid reserves and activity of pesticide detoxification enzymes, such as esterases. Krischik et al. (2015) identified no acute lethal effects of neonicotinoid contamination of nectar resources on adult monarchs, but further research is needed to evaluate chronic and sublethal effects, including from potentially synergistically toxic combinations of systemic fungicides and neonicotinoids (e.g., Tsvetkov et al. 2017).

Implications for conservation

We identify several KDEM core migratory pathways in which to focus conservation efforts for supporting adult fall migrating monarchs based upon our spatial hazard analysis (c.f., Oberhauser et al. 2017). The Central and Coastal funnels should be the focus for further investigation of potential impacts and mitigation for fall monarch roadkill. Coastal portions of the Eastern Flyway would most benefit from wildflower plantings (e.g., Venturini et al. 2017), perhaps in association with roosting areas and away from roads to mitigate potential negative impacts from both mosquito truck ULV spraying and roadkill. Intensely cropped regions of the core Central Flyway migratory pathway in the Midwest are the most important for studying potential benefits of providing additional nectar resources that are also free of pesticide contamination. The MaxEnt model also supports the importance of conserving nectar resources found in grassland habitats of the Great Plains, including throughout the Central Funnel of Oklahoma and Texas. Brower et al. (2015) suggested that nectar sources of Texas and North Mexico are especially crucial for the fall migration (also see Inamine et al. 2016). Additional potential fall adult migratory threats also need further investigation, such as how much the interaction of migrants and coastal resident monarchs breeding on tropical milkweed (*Asclepias curassavica*) impacts parasitism of migrants by *Ophryocystis elektroscirrha* (Satterfield et al. 2018). The tendency of the Central Funnel to shift as much as 200 km in some years, especially towards the West, has important implications for where conservation efforts to support the fall migration should be focused. Spatial risk assessment is needed to determine the degree of exposure to identified potential anthropogenic hazards along the monarch core fall migration pathways. The proposed mitigation strategies for identified threats along the core pathways can provide regional guidance for developing future conservation plans and risk assessments in the continued effort to preserve the unique phenomenon of the eastern monarch fall migration (embedded zipped shapefiles of core migration pathways and potential hazards are provided in Table B.4).

Conclusion

The two fundamentally different approaches of interpolative spatial point pattern KDEMs and correlative ecological niche models, such as MaxEnt, can provide valuable complementary perspectives on animal migration pathways. We made several novel applications of spatially interpolative KDEMs for identifying broad spatial patterns of migratory movement, revealing their value for modeling movement through areas that may be of low environmental suitability. A consensus of KDEMs developed from sets of training data across all years was useful in distinguishing broad scale migratory flyways. Annual KDEMs are useful for investigating yearly variability in migratory movement patterns, and the consensus of combined annual KDEMs is effective in delineating narrower core migration pathways over several years. Correlative niche models can reveal the most environmentally suitable habitats along the migratory pathway. The combination of these approaches facilitates spatially identifying migratory hazards which can be used in migratory risk assessments and conservation planning for declining migratory species.

The benefits of utilizing the two migration modeling approaches were demonstrated in developing fall migration models for the eastern population of monarch butterflies. The various KDEMs were best suited in defining the entire fall migration pathway, and revealing core migratory pathways. The MaxEnt model best revealed the alignment of the migration pathway of the Central Flyway with nectar resources in the Great Plains grasslands. The overlap of the identified core monarch migration pathways with potential anthropogenic hazards of roadkill, mosquito adulticide ULV spray exposure, and the loss and contamination of nectar resources due to agricultural pesticides can help focus future conservation activities and risk assessments. This study additionally affirms the high value of citizen science efforts, such as Journey North, in understanding the eastern monarch migration. Complementary approaches such as KDEMs and correlative niche modeling should be further explored for migration modeling of monarchs and other migratory animals of conservation concern. Potential hazards and conservation strategies identified for the eastern monarch core fall migratory pathways deserve further investigation.

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Figure Captions

Fig. 1. Monarch overnight roosts from 2002 to 2016 (Journey North 2017), range of the monarch eastern migratory population, previous and updated monarch flyway divisions, background evaluation extent for model training and testing, migration pathway of minimum consensus kernel density estimation model (KDEM) with north (37-50°N) and south (27-37°N) centroids for the KDEM in the Central Flyway (see Table B.4 for shapefiles). *[Color Figure Online]*

Fig. 2. Monarch fall migration pathway minimum calibration consensus of average consensus models for 2002–2016, including CEC (2005) ecoregions (including 100% consensus boundaries): (A) MaxEnt feature subset ensemble of 12 models developed from subsets of six of 80 variables by random subset feature selection for high AUC_{psa} ; and (B) kernel density estimation model (KDEM) training set ensemble of three models developed by three-fold training data partition (see Table B.4 for shapefiles; see Figs. 1 and 5A for binary minimum consensus KDEM). *[Color Figure Online]*

Fig. 3. Evaluation statistics of MaxEnt niche model versus kernel density estimation model (KDEM) of monarch fall migration pathways (mean \pm SD): (A) AUC_{psa} , (B) $AICc$, and (C) AUC_{psa_diff} (overfitting) (see Fig. 2 for models). Means with an asterisk for a statistic are significantly more optimal (higher for AUC_{psa} and lower for $AICc$ and AUC_{psa_diff}) ($P < 0.05$; Welch t test). *[Color Figure Online]*

Fig. 4. Monarch fall migration pathway kernel density estimation models (KDEMs) from (A) 2002–2016 combined data, and (B–M) for each year from 2005 to 2016. Models represent minimum frequency consensus training set ensemble of three binary calibrated models developed from a three-fold training data partition. *[Color Figure Online]*

Fig. 5. Monarch fall migration pathway 100% consensus boundary of annual kernel density estimation models (KDEMs) for 2005-2016 ($n = 12$, blue shading; see Fig. 4 for individual annual KDEMs) and potential anthropogenic hazards: (A) monarch roadkill hotspots (Table SB.5, Fig. B.6), monarch mortality from late season mosquito adulticide ultra-low volume (ULV) spray treatments (Table B.6), months for latest ULV spraying (Table B.7), approximate dates for peak migration along the Central Flyway (pink text) and Eastern Flyway (blue text) (Calvert and Wagner 1999; Howard and Davis 2009), and US counties with ULV spraying; (B) US counties with 75th percentile level uses of glyphosate herbicide and neonicotinoid insecticides (clothianidin, imidacloprid, and thiamethoxam) in 2014 (see Table B.4 for shapefiles). *[Color Figure Online]*

Figures

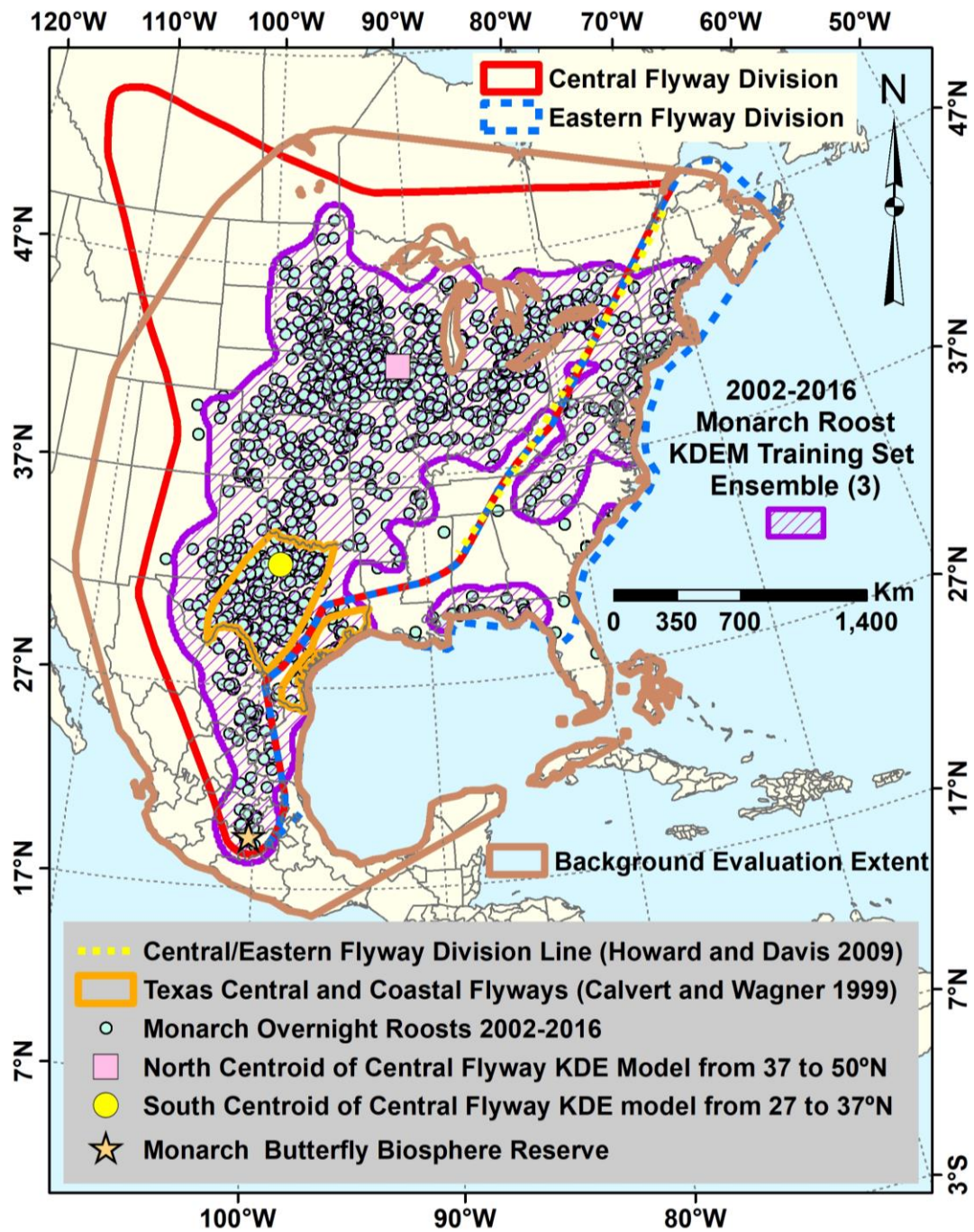


Fig. 1

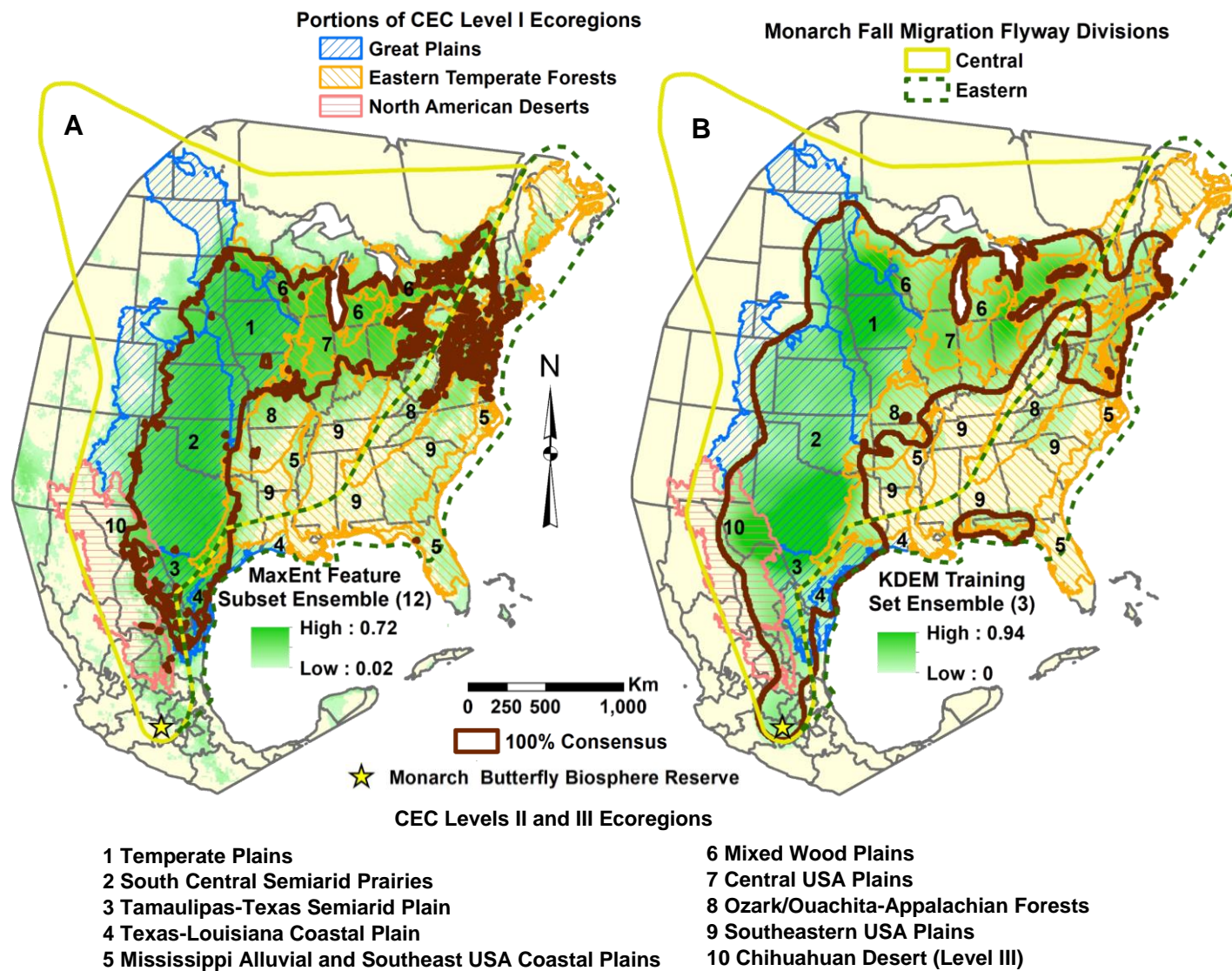


Fig. 2

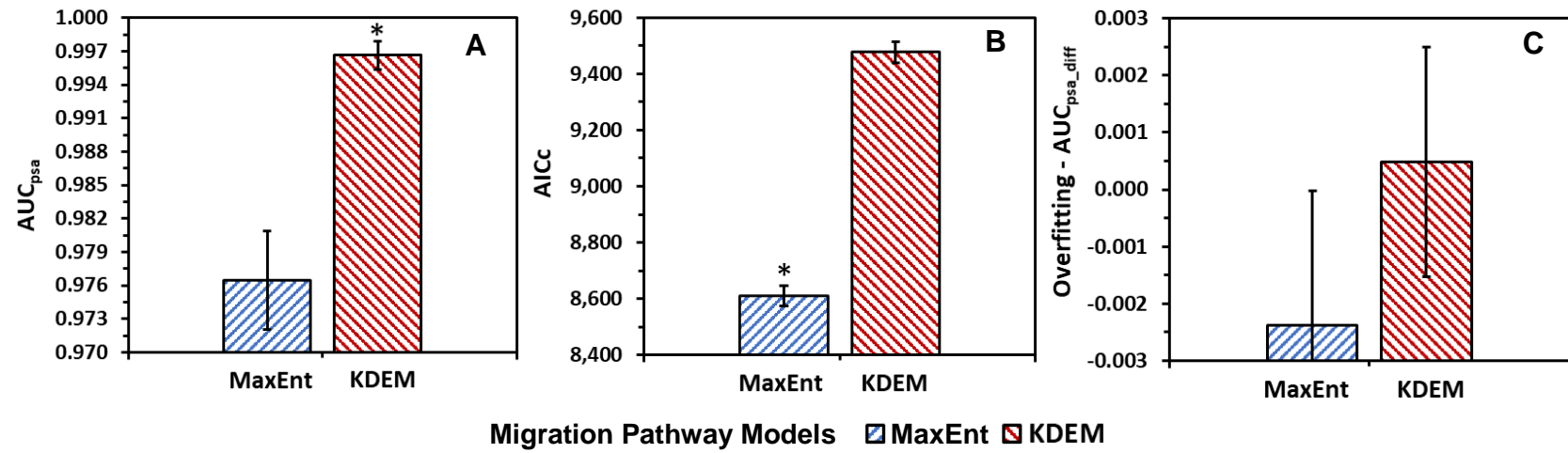


Fig. 3

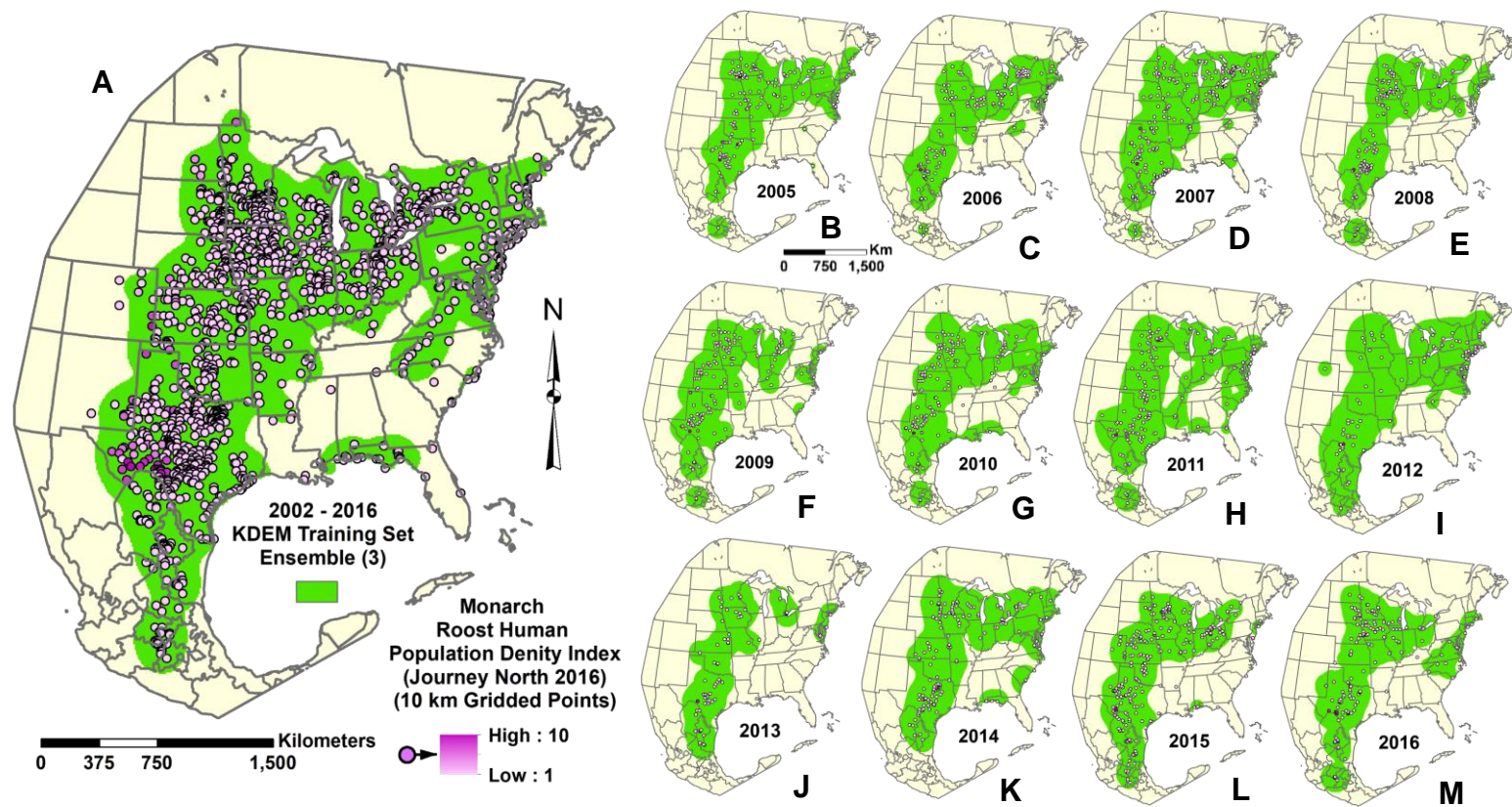


Fig. 4

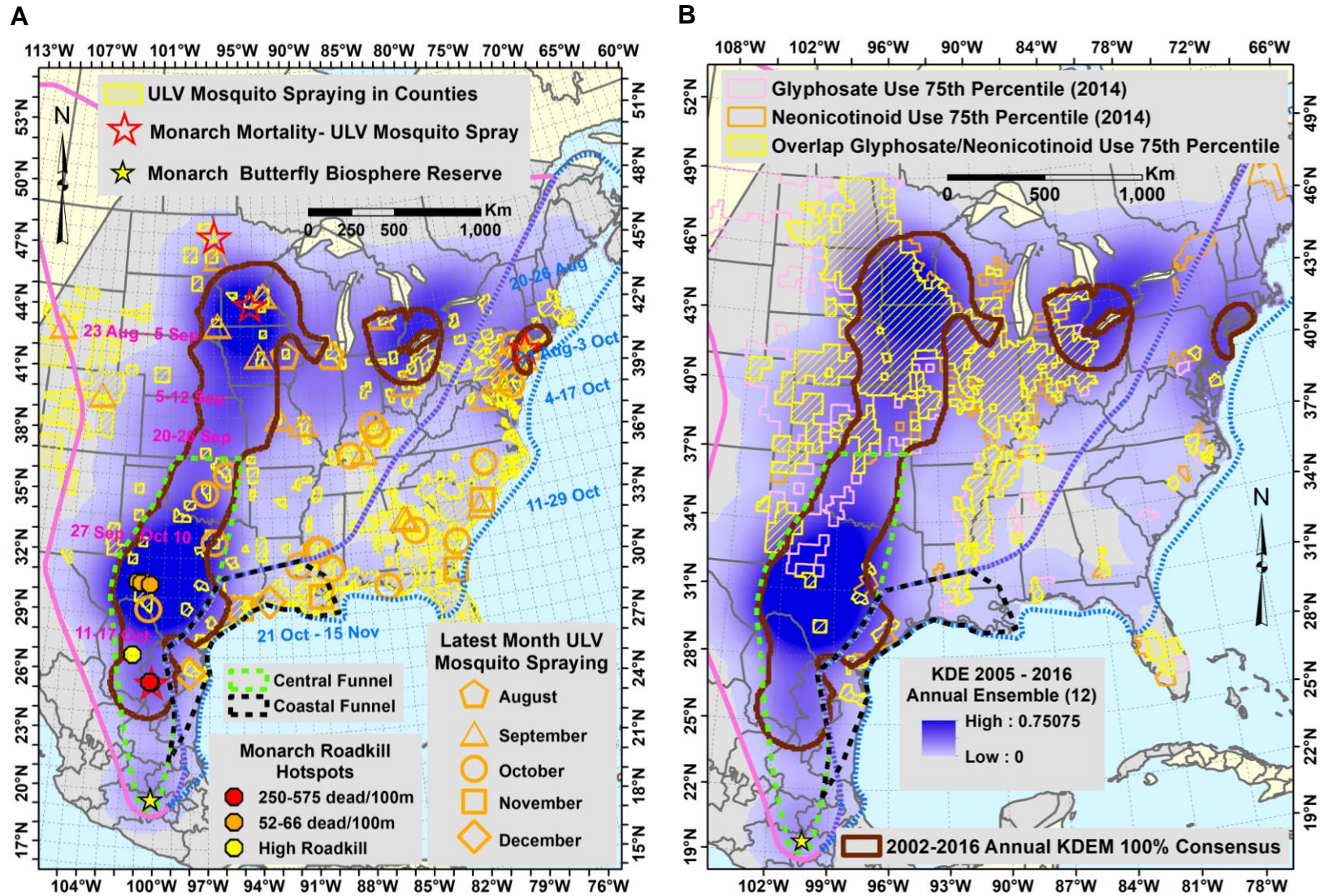


Fig. 5

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APPENDIX B
MANUSCRIPT SUPPLEMENT:
MODELING FALL MIGRATION PATHWAYS AND SPATIALLY
IDENTIFYING POTENTIAL MIGRATORY HAZARDS FOR THE EASTERN
MONARCH BUTTERFLY

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Supplementary Material

Methods

MaxEnt migration pathways

Feature selection

In accordance with standard feature selection terminology (e.g., Jović et al. 2015), we refer to environmental variables (predictors) as “features”, distinguishing them from the multiple MaxEnt “features” derived from individual environmental variables (e.g., various quadratic and hinge “features”), which we refer to as “derived features”. The random subset feature selection algorithm (RSFSA) is an ensemble hybrid filter/wrapper feature selection algorithm (FSA) (c.f., Jović et al. 2015) that evaluates features as an entire group (feature subset) in niche models. The filter and wrapper are two types of search criteria, representing two RSFSA phases, for selecting feature subsets for developing higher performance MaxEnt models. The “filter” criterion is not derived from the classification algorithm (i.e., MaxEnt; c.f. Jović et al. 2015) and is represented by a correlation threshold limiting the absolute value of Spearman’s rank correlation coefficient ($|r_s|$) among a feature subset to 0.7 (Dormann et al. 2013). The correlation filter is employed in randomly generating thousands of feature subsets through multiple random sequential forward selection. The “wrapper” criterion, by which RSFSA is “wrapped” with the classification algorithm (c.f. Jović et al. 2015), is represented by either the AUC accuracy statistic or AICc information criterion, which is calculated for MaxEnt models developed for each of the thousands of random feature subsets. These wrappers are used to rank the thousands of MaxEnt models by AUC and AICc. Subset feature selection allows identification of potentially synergistic combinations of features that can be missed using sequential selection algorithms that are typically used for niche models, which evaluate single features at a time (Tracy et al. 2018).

The RSFSA is basically a brute-force approach to screening thousands of niche models for better performance that requires much computation time, but which is reduced by parallel processing. Derived features are not selected by RSFSA, but they are screened by lasso L1 regularization which is an embedded FSA within the MaxEnt algorithm (Phillips and Dudik 2008). Model training and testing data held out from the RSFSA MaxEnt wrapper ranking is used to evaluate the RSFSA-selected feature subset ensemble (FSE) against an FSE of random subsets for improvement of AUC, AICc, and AUC_{DIFF}. In order to estimate the smallest feature subset size between one and twenty features beyond which increasing the number of features produces negligible gains in model performance (RSFSA Stage I), the top ten ranked MaxEnt models developed from about 300 feature subsets for each feature subset size were compared in performance. Once an optimal feature subset size was estimated, three training/testing data randomization RSFSA replicates, each consisting of 3,000 MaxEnt model FSEs (making 9,000 models total), were ranked by both AUC or AICc (whichever is most consistent in selecting higher performance models among the randomizations) to select the top 12 MaxEnt models (RSFSA Stage II; for further details, see Tracy et al. 2018).

Joint ranking of environmental variables employed in the top 12 selected models using weighted criteria (0.6 for mean variable permutation importance; 0.4 for frequency of variable appearance in top 12 models) was performed with a Multi Multi-Objective Optimization Ratio Algorithm (plus Full Multiplicative Form, MMOORA) of Brauers and Zavadskas (2010), which

is implemented in the MCDM R package (Ceballos Martín 2016).

Kernel density estimation model migration pathways

To calculate the monarch roost human population density index, the raw roost data was first converted to a 10 km resolution raster with values of one for roost presence and “no data”. This process produced one roost record per 10 km grid cell. The 5 km resolution human population density grid (CIESIN 2005) was aggregated to 10 km resolution to match the resolution of the occurrence data. A focal mean of population density was then calculated within 30 by 30 km windows. The roost presence raster (values of one where present) was divided by the population density raster to yield a raw monarch roost by human population density index, with smaller numbers indicating higher populations. In order to restrain the influence of the index, values of the raw human population density index that were below one were changed to one, and values above ten were limited to ten, yielding a monarch roost human population density index raster. The raster was converted to a point shapefile with values ranging continuously from one in densely populated areas (most points) to 10 in sparsely populated areas. The KDE surface was then calculated based on this shapefile, with the index limits of one to ten constraining the maximum influence of human population density to a factor of 10.

Flyways and core migratory pathways

Flyway division boundaries within the larger monarch eastern migratory population from Canada to Mexico were updated using the migration pathways identified by a minimum consensus ensemble of the combined 2002 to 2016 KDE model (KDEM) training set ensembles (TSEs) and individual roost records. Core fall migration routes, including core pathways through the narrower southern funnel portions, were also identified by generally following the boundaries of flyways and the 100% consensus boundary of the annual KDEM ensemble (see Table B.4 for shapefiles).

Anthropogenic fall migration hazards

Eastern US counties in which mosquito abatement programs occur that potentially spray ultra-low volume (ULV) adulticides in the late season were identified from a variety of screened governmental and news internet sources which we assembled as a form of citizen science data. These sources include data from states (Delaware Department of Natural Resources 2012; Florida Medical Entomology Laboratory 2018; Georgia Mosquito Control Association 2018; Kentucky Department of Agriculture 2018; Louisiana Mosquito Control Association 2018; Maryland Department of Agriculture 2018; www.Mass.gov 2018; Michigan Mosquito Control Association 2018; Mississippi State Department of Health 2018; New Jersey Department of Environmental Protection 2018; Pennsylvania West Nile Virus Control Program 2018; South Carolina Mosquito Control Association 2018; Texas Mosquito Control Association 2018; Vector Disease Control International 2018), counties, and cities (e.g., Table B.7).

Results

Monarch migration pathways

Feature selection

Stage I of the RSFSA was used to identify six out of 80 variable subsets as optimal for maximizing accuracy (AUC_{psa}), minimizing complexity ($AICc$), and minimizing overfitting (AUC_{psa_diff}) in MaxEnt migration models (Fig. B.1A-C). In RSFSA Stage II, the top 250 of 3,000 six-variable MaxEnt models selected by AUC_{psa} (rather than $AICc$) exhibited significantly higher AUC_{psa} and lower $AICc$ than 300 random MaxEnt models for all three training data set replications (Fig. B.1D,E). Significantly lower overfitting (AUC_{psa_diff}) was only evident for AUC_{psa} selected MaxEnt models in one replication (Fig. B.1F). Out of 9,000 randomly generated six-variable MaxEnt models we selected the top 12 as ranked by AUC_{psa} for inclusion in a feature subset ensemble model (Fig. 2A).

Kernel density estimation model migration pathways

In comparison to KDEMs that did not account for human population density related observer bias (Fig. B.3A), the KDEMs accounting for human population density appeared to project much reduced, but not eliminated, bias towards higher roost densities around metropolitan areas, such as Minneapolis/St Paul, Dallas/Fort Worth, and Austin/San Antonio (Fig. B.3B). However, differences in the KDEM 100% consensus boundaries for the two models appear to be less pronounced (Fig. B.3A,B).

Annual kernel density estimation model migration pathways

The average width of the kernel density estimation model (KDEM) migration pathway in the northern portion of the Central Flyway (37-50°N latitude, from northern Oklahoma border northwards) varied by 662 km, ranging from 694 km in 2013 to 1,356 km in 2007 (Fig. B.4A). The average width of the KDEM migration pathway in the southern portion of the Central Flyway (27-37°N) varied by 392 km, ranging from 519 km in 2013 to 910 km in 2011 (Fig. B.4D). The annual north to south shift from average for the northern portion of the KDEM migration pathway in the Central Flyway varied by 143 km, ranging from 73 km to the south in 2015 to 70 km to the north in 2012 (Fig. B.4B). The annual north to south shift from average for the southern portion of the KDEM migration pathway in the Central Flyway varied by 131 km, ranging from 111 km to the south in 2006 to 19 km to the north in 2007 (Fig. B.4E). The annual east to west shift from average for the northern portion of the KDEM migration pathway in the Central Flyway varied by 353 km, ranging from 134 km to the west in 2016 to 220 km to the east in 2006 (Fig. B.4C). The annual east to west shift from average for the southern portion of the KDEM migration pathway in the Central Flyway varied by 306 km, ranging from 207 km to the west in 2015 to 99 km to the east in 2007 (Fig. B.4F).

Flyways and core migratory pathways

Guided by the 100% consensus 2002-2016 KDEM, we extended the boundary division between the Central Flyway and Eastern Flyway of Howard and Davis (2009) to proceed southwest from Alabama to the border of eastern Texas (Fig. 1). Through Texas, we continued the division boundary by following a line bisecting the Central and Eastern (Coastal) Flyways, as identified by

Calvert and Wagner (1999). We extended the division boundary from the Texas border towards the Monarch Butterfly Biosphere Reserve in Central Mexico by generally following the eastern boundary of roost records.

The 100% consensus of the 2002-2016 combined data KDEM outlines a fairly broad migration pathway extending from the Great Lakes region to northeastern Mexico, and it includes portions of the Eastern Flyway (Figs. 1, B.3B). In contrast, the 100% consensus of 2005-2016 annual KDEMs encompasses a narrower pathway that is primarily restricted to the Central Flyway division, with the exception of the Texas-Louisiana Coastal Plain (Figs. 5, B.3C). The narrower 100% consensus annual KDEM was used to define the core migratory pathway and guide the delineation of southern core migratory pathway funnels for each of the two flyway divisions (Figs. 5, B.3C). The Central Funnel was defined along the 100% consensus annual KDEM in the Central Flyway from Oklahoma to the Mexican overwintering sites. The Coastal Funnel was defined within the Eastern Flyway from southern Louisiana along the coastal region of Texas to Northeast Mexico where it was merged with the Central Funnel (Figs. 5, B.2, B.3C).

Discussion

Flyways and core migratory pathways

The KDEMs indicate the Central Flyway as the primary route of the fall monarch migration (Figs. 1, 2B, 5, B.3B-C). Few sites with regular fall accumulations and roosting currently exist in the Eastern Flyway (Garland and Davis 2002). Howard and Davis (2009) hypothesized that smaller roost sizes in the southern portion of the Eastern Flyway may result in roosts being less noticed, leading to fewer reports from this area. Further studies of regional variations in roost densities across the two flyways are needed, especially for the Coastal Funnel in Mexico where data are totally lacking. The southern narrower Central Funnel and Coastal Funnel pathways (Figs. 5, B.2, B.3C) can serve to focus further research into conservation planning for the fall monarch migration.

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Tables

Table B.1. Eastern monarch fall overnight roost records from 2002-2016 (Journey North 2017).

Year	Observations	Earliest Day of Year	Latest Day of Year	Duration (Days)
2002	25	239	309	70
2003	103	215	321	106
2004	70	214	314	100
2005	189	227	305	78
2006	173	223	360	137
2007	269	217	360	143
2008	136	222	312	90
2009	160	228	311	83
2010	336	223	315	92
2011	185	314	314	91
2012	138	312	312	76
2013	109	242	317	75
2014	245	236	340	104
2015	422	227	339	115
2016	243	234	335	121
Total	2803			
Mean \pm SD per				
Year	186.9 \pm 103.4	238.2 \pm 31.5	324.3 \pm 18.1	98.7 \pm 22.4

Table B.2. Eighty environmental predictor indices (10 km resolution) used in developing 12 selected MaxEnt monarch overnight roost niche models with six of 80 variables.

Variable Index (Source)	Grid Name Abbreviation	Variable Frequency in 12 Selected Models (% of 72) ^a
57 Climatic Indices (for 1960–1990 derived from WorldClim [2014] of Hijmans et al. [2005])		
19 Bioclim Indices (WorldClim 2014)		
Annual mean temperature	<i>bio_1</i>	0 (0.0)
Mean diurnal range (mean of monthly TMAX – TMIN)	<i>bio_2</i>	0 (0.0)
Isothermality (<i>bio_2/bio_7</i>) ($\times 100$)	<i>bio_3</i>	0 (0.0)
Temperature seasonality (standard deviation $\times 100$)	<i>bio_4</i>	0 (0.0)
Maximum temperature of warmest month	<i>bio_5</i>	0 (0.0)
Minimum temperature of coldest month	<i>bio_6</i>	0 (0.0)
Temperature annual range (<i>bio_5 – bio_6</i>)	<i>bio_7</i>	0 (0.0)
Mean temperature of wettest quarter	<i>bio_8</i>	0 (0.0)
Mean temperature of driest quarter	<i>bio_9</i>	0 (0.0)
Mean temperature of warmest quarter	<i>bio_10</i>	2 (2.8)
Mean temperature of coldest quarter	<i>bio_11</i>	0 (0.0)
Annual precipitation	<i>bio_12</i>	0 (0.0)
Precipitation of wettest month	<i>bio_13</i>	0 (0.0)
Precipitation of driest month	<i>bio_14</i>	0 (0.0)
Precipitation seasonality (coefficient of variation)	<i>bio_15</i>	6 (8.3)
Precipitation of wettest quarter	<i>bio_16</i>	1 (1.4)
Precipitation of driest quarter	<i>bio_17</i>	1 (1.4)
Precipitation of warmest quarter	<i>bio_18</i>	0 (0.0)
Precipitation of coldest quarter	<i>bio_19</i>	1 (1.4)
Subtotal		11 (15.3)
19 Supplementary Climatic (SuppClim) Indices^{b,c}		
Annual mean minimum temp. of coldest month (TMIN)	<i>tmin_ann</i>	0 (0.0)
	<i>tmin_winq,</i>	0 (0.0)
	<i>tmin_sprq,</i>	4 (5.6)
	<i>tmin_sumq,</i>	0 (0.0)
Quarterly mean monthly minimum temperature (4)	<i>tmin_autq</i>	2 (2.8)
Annual mean maximum temp. of warmest month (TMAX)	<i>tmax_ann</i>	0 (0.0)

Table B.2 (cont.). Eighty environmental predictor indices (10 km resolution) used in developing 12 selected MaxEnt monarch overnight roost niche models with six of 80 variables.

Variable Index (Source)	Grid Name Abbreviation	Variable Frequency in 12 Selected Models (% of 72) ^a
	<i>tmax_winq,</i>	0 (0.0)
	<i>tmax_sprq,</i>	1 (1.4)
	<i>tmax_sumq,</i>	1 (1.4)
Quarterly mean monthly maximum temperature (4)	<i>tmax_autq</i>	0 (0.0)
Annual mean monthly rainfall (P) (mm)	<i>prec_ann</i>	0 (0.0)
	<i>prec_winq,</i>	0 (0.0)
	<i>prec_sprq,</i>	4 (5.6)
	<i>prec_sumq,</i>	1 (1.4)
Quarterly mean monthly rainfall (50%) (4)	<i>prec_autq</i>	0 (0.0)
Effective Warmth Index (from mean monthly temperatures $\times 10$ above 5°C)	<i>ew_indx</i>	1 (1.4)
Rivas-Martinez (RM) ombrothermic index (from monthly MTMP and P)	<i>ombro_index</i>	1 (1.4)
RM continentality index (TMAX – TMIN) $\times 10$ (CONT)	<i>cont_index</i>	0 (0.0)
RM thermicity index (MTMP + TMX_COLD + TMN_COLD) $\times 10$	<i>therm_index</i>	0 (0.0)
Subtotal		15 (20.8)
<i>19 Actual and Potential Evapotranspiration (AET-PET) Indices</i> (PET; Zomer et al. 2007; 2008; AET; Trabucco and Zomer 2010) ^c		
Total annual reference evapotranspiration from Hargreaves model (PETH) (mm)	<i>tpeth_ann</i>	0 (0.0)
	<i>peth_winq,</i>	0 (0.0)
	<i>peth_sprq,</i>	1 (1.4)
	<i>peth_sumq,</i>	1 (1.4)
Quarterly mean monthly PETH (4)	<i>peth_autq</i>	0 (0.0)
Thornwaite summer concentration thermal efficiency (summer PETH/annual PETH) $\times 1000$	<i>tpeths_tpetha</i>	0 (0.0)
Willmott and Feddema climate moisture index (from total annual PETH and PREC) $\times 1000$	<i>im_index</i>	0 (0.0)
Total annual actual evapotranspiration from Thornwaite-Mather water balance model (TMWBM) (AETT) (mm)	<i>taett_tann</i>	4 (5.6)

Table B.2 (cont.). Eighty environmental predictor indices (10 km resolution) used in developing 12 selected MaxEnt monarch overnight roost niche models with six of 80 variables.

Variable Index (Source)	Grid Name Abbreviation	Variable Frequency in 12 Selected Models (% of 72) ^a
	<i>aett_winq</i> ,	1 (1.4)
	<i>aett_sprq</i> ,	2 (2.8)
	<i>aett_sumq</i> ,	0 (0.0)
Quarterly mean monthly AETT (4)	<i>aett_autq</i>	4 (5.6)
Total annual evapotranspiration ratio (AETT/PETH) × 10	<i>etr_t_ann</i>	1 (1.4)
	<i>etr_t_winq</i> ,	0 (0.0)
	<i>etr_t_sprq</i> ,	1 (1.4)
	<i>etr_t_sumq</i> ,	0 (0.0)
Quarterly mean monthly AETT/PETH (4) × 1000	<i>etr_t_autq</i>	0 (0.0)
Modified Driscoll-Yee Fong Continentality index ^d	<i>cont_dfmo</i>	4 (5.6)
Climate water deficit (<i>tpeth_ann</i> – <i>taett_tann</i>)	<i>cwd_ann</i>	2 (2.8)
Subtotal		21 (29.2)
12 Topographic Indices		
<i>Eight Geomorphologic Indices</i> (derived from 15 arc second resolution HydroSHEDs grids of Lehner et al. 2008; last six indices calculated using Geomorphometry and Gradient Metrics Toolbox for ArcGIS [Evans et al. 2014]) ^c		
Elevation	<i>elev</i>	0 (0.0)
Slope	<i>slope</i>	1 (1.4)
Topographic Position Index (TPI), 19 km circular radius	<i>tpi19kr</i>	1 (1.4)
Compound Topographic Index (CTI)	<i>cti</i>	1 (1.4)
Heat Load Index (HLI)	<i>hli</i>	0 (0.0)
Integrated Moisture Index (IMI)	<i>imi</i>	3 (4.2)
Site Exposure Index (SEI)	<i>sei</i>	1 (1.4)
Slope Cosine Aspect Index (SCAI)	<i>scai</i>	1 (1.4)
<i>Four Hydrogeomorphologic Indices</i> (derived from 15 arc second resolution HydroSHEDs polyline river network shapefile of Lehner et al. 2008)		
Distance to Streams (STRMDIST)	<i>strmdist</i>	0 (0.0)
Distance to Low Flow Accumulation Areas (< 5,000 cells; STRMLOFLODIST)	<i>strmloflodist</i>	3 (4.2)
Distance to Medium Flow Accumulation Areas (5,000–60,000 cells; STRMMDFLODIST)	<i>strmmdflodist</i>	1 (1.4)

Table B.2 (cont.). Eighty environmental predictor indices (10 km resolution) used in developing 12 selected MaxEnt monarch overnight roost niche models with six of 80 variables.

Variable Index (Source)	Grid Name Abbreviation	Variable Frequency in 12 Selected Models (% of 72) ^a
Distance to High Flow Accumulation Areas (>60,000 cells; STRMHIFLODIST)	<i>strmhiflodist</i>	3 (4.2)
Subtotal		15 (20.8)
11 Land Cover Indices (percent cover per 1 km ² for 2001 to 2005 from Tuanmu and Jetz [2014] and EarthEnv [2016])		
Cultivated and Managed Vegetation	<i>agric_lc</i>	1 (1.4)
Barren	<i>barren_lc</i>	1 (1.4)
Deciduous Broadleaf Trees	<i>decbrdtree_lc</i>	1 (1.4)
Evergreen Broadleaf Trees	<i>evgbrdtree_lc</i>	1 (1.4)
Regularly Flooded Vegetation	<i>floodveg_lc</i>	1 (1.4)
Herbaceous Vegetation	<i>herb_lc</i>	1 (1.4)
Mixed/Other Trees	<i>mixothtree_lc</i>	1 (1.4)
Evergreen/Deciduous Needleleaf Trees	<i>needletree_lc</i>	1 (1.4)
Shrubs	<i>shrub_lc</i>	0 (0.0)
Urban/Built-up	<i>urban_lc</i>	1 (1.4)
Open Water	<i>water_lc</i>	1 (1.4)
Subtotal		10 (13.9)

^aFrequency out of six variables times 12 models = 72 instances.

^bQuarters: winter – Jan, Feb, Mar; spring – Apr, May, Jun; summer – Jul, Aug, Sep; autumn – Oct, Nov, Dec. PREC = precipitation; TMAX = mean temperature of warmest month; TMIN = mean temperature of coldest month; TMAX_COLD = mean maximum temperature of coldest month; TMN_COLD = mean minimum temperature of coldest month.

^cFor additional sources of indices and details see Tracy et al. (2018).

^dIncorporates moisture correction factor of AET/PET × 10.

Table B.3. MaxEnt model variable permutation importance for 42 of 80 variables used in top 12 six-variable monarch overnight roost models selected by random subset feature selection algorithm.

Variable ^a	MaxEnt Model Permutation Importance, Mean \pm SD (number of top 12 models)	Multi Multi-Objective Optimization Ranking by Mean Permutation Importance (0.6 weight) and Number Appearances in Top 12 Models (0.4 weight) ^b
<i>tmin_sprq</i>	54.8 \pm 4.2 (4)	1
<i>aett_autq</i>	36.3 \pm 3.2 (4)	2
<i>taett_tann</i>	25.3 \pm 1.3 (4)	3
<i>prec_sprq</i>	16.0 \pm 1.5 (4)	4
<i>tmin_autq</i>	47.6 \pm 9.1 (2)	5
Precipitation seasonality (<i>bio_15</i>)	9.5 \pm 7.2 (6)	6
Mean temperature of warmest quarter (<i>bio_10</i>)	36.9 \pm 6.2 (2)	7
<i>cont_dfmo</i>	10.0 \pm 8.5 (4)	8
<i>aett_sprq</i>	25.0 \pm 4 (2)	9
<i>cwd_ann</i>	14.8 \pm 3.8 (2)	10
<i>ew_indx</i>	54.0 \pm 0 (1)	11
<i>aett_winq</i>	35.8 \pm 0 (1)	12
<i>ombro_index</i>	31.7 \pm 0 (1)	13
<i>tmax_sumq</i>	31.0 \pm 0 (1)	14
<i>etrt_ann</i>	24.6 \pm 0 (1)	15
<i>etrt_sprq</i>	23.4 \pm 0 (1)	16
<i>strmhiflodist</i>	0.2 \pm 0.1 (3)	17
<i>strmloflodist</i>	0.2 \pm 0.1 (3)	18
<i>imi</i>	0.1 \pm 0 (3)	19
Precipitation of coldest quarter (<i>bio_19</i>)	19.0 \pm 0 (1)	20
Precipitation of driest quarter (<i>bio_17</i>)	17.8 \pm 0 (1)	21
<i>peth_sprq</i>	15.6 \pm 0 (1)	22
<i>tmax_sprq</i>	14.3 \pm 0 (1)	23
Precipitation of wettest quarter (<i>bio_16</i>)	12.3 \pm 0 (1)	24
<i>peth_sumq</i>	12.1 \pm 0 (1)	25
<i>prec_sumq</i>	11.4 \pm 0 (1)	26
<i>decbdtree_lc</i>	11.3 \pm 0 (1)	27
<i>mixothtree_lc</i>	4.3 \pm 0 (1)	28
<i>needletree_lc</i>	1.4 \pm 0 (1)	29
<i>urban_lc</i>	1.2 \pm 0 (1)	30
<i>slope</i>	1.1 \pm 0 (1)	31

Table B.3 (cont.). MaxEnt model variable permutation importance for 42 of 80 variables used in top 12 six-variable monarch overnight roost models selected by random subset feature selection algorithm.

Variable ^a	MaxEnt Model Permutation Importance, Mean \pm SD (number of top 12 models)	Multi Multi-Objective Optimization Ranking by Mean Permutation Importance (0.6 weight) and Number Appearances in Top 12 Models (0.4 weight) ^b
<i>agric_lc</i>	0.7 \pm 0 (1)	32
<i>scai</i>	0.3 \pm 0 (1)	33
<i>cti</i>	0.1 \pm 0 (1)	34
<i>evgbrdtree_lc</i>	0.1 \pm 0 (1)	35
<i>barren_lc</i>	0.1 \pm 0 (1)	36
<i>herb_lc</i>	0.1 \pm 0 (1)	37
<i>water_lc</i>	0.0 \pm 0 (1)	38
<i>sei</i>	0.0 \pm 0 (1)	39
<i>strmmdflodist</i>	0.0 \pm 0 (1)	40
<i>floodveg_lc</i>	0.0 \pm 0 (1)	41
<i>tpi19kr</i>	0.0 \pm 0 (1)	42

^aSee Table B.2 for variable abbreviations and sources of variables.

^bVariables ranked using weighted joint criteria with MCDM R package.

Table B.4. Shapefiles of geographic information system (GIS) layers developed in eastern monarch butterfly fall migration study (see Methods for additional details and sources).








GIS Layers (Source)	Shapefile (Zipped)
Monarch Flyways (This Study)	
Monarch Eastern Flyway (Fig. 1, B.2)	 MonarchEasternFlywayShapefile.zip
Monarch Central Flyway (Fig. 1, B.2)	 MonarchCentralFlywayShapefile.zip
MaxEnt and Kernel Density Estimation Model (KDEM) Migration Pathways from Monarch Roosts (Journey North 2017, This Study)	
Monarch Roost 2002-2016 MaxEnt Feature Subset Ensemble 100% Consensus Boundary (Fig. 2A)	 MonarchRoost2002_2016CombinedMaxEntConsensusShapefile.zip
Monarch Roost 2002-2016 KDEM Training Set Ensemble 100% Consensus Boundary (Figs. 2B, B.3B)	 MonarchRoost2002_2016CombinedKDEMConsensusShapefile.zip
Monarch Roost 2002-2016 KDEM Training Set Ensemble Binary Minimum Consensus Boundary (Figs. 1, 4A)	 MonarchRoost2002_2016CombinedBinaryMinimumConsensusShapefile.zip
Monarch Roost 2005-2016 KDEM Annual Ensemble 100% Consensus Boundary (Figs. 5, B.3C)	 MonarchRoost2005_2016AnnualKDEMConsensusShapefile.zip
Monarch Southern Core Migration Pathways (This Study)	
Monarch Central Funnel (Figs. 5, B.2, B.3C)	 MonarchCentralFunnelShapefile.zip

Table B.4 (cont.). Shapefiles of geographic information system (GIS) layers developed in eastern monarch butterfly fall migration study (see Methods for additional details and sources).

GIS Layers (Source)	Shapefile (Zipped)
Monarch Coastal Funnel (Figs. 5, B.2, B.3C)	MonarchCoastalFunnelShapefile.zip
Spatially Identified Potential Migratory Hazards (This Study)	
Monarch Roadkill Hotspots (Figs. 5A, B.6A)	ReportedMonarchRoadkillHotspots.zip
Locations for Monarch Mortality from Ultra-Low Volume (ULV) Mosquito Control Spraying (Fig. 5A)	MonarchMortalityMosquitoULVFallTreatments.zip
Latest Fall ULV Spraying in Eastern US Cities and Counties (Fig. 5A)	MosquitoLatestULVMonthTreatments.zip
Eastern US Counties with Ultra-Low Volume (ULV) Mosquito Control Treatments (Fig. 5A)	EasternMonarchMosquitoControlAlb.zip
Upper 75 th Percentile of 2014 Glyphosate Use in US Counties (imidacloprid, clothianidin, and thiamethoxam) (Fig. 5B)	GlyphosateUse2014Counties75thPerc.zip
Upper 75 th Percentile of 2014 Neonicotinoid Use in US Counties (imidacloprid, clothianidin, and thiamethoxam) (Fig. 5B)	NeonicotinoidUse2014Counties75thPerc.zip
Overlap in Upper 75 th Percentile of 2014 Glyphosate Use in US Counties (imidacloprid, clothianidin, and thiamethoxam) and 2014 Neonicotinoid Use in US Counties (imidacloprid, clothianidin, and thiamethoxam) (Fig. 5B)	GlyphosateNeonicotinoidsOverlap75thPerc.zip

Table B.5. Monarch roadkill hotspot locations.

Location	Latitude	Longitude	Date	Monarchs Dead per Single Roadside Edge	Comments	Source
Mexico 40D, southwest of Monterrey	25.67413	-100.50173	31-Oct-15	115/20 m (575/100m)	Coordinates based on photo and Google Earth Street View	Ana Velia Rodríguez de Silva in Correo Real (2015) ^a
Mexico 40D, southwest of Monterrey	25.69623	-100.58511	31-Oct-15	10/4m (250/100m)	Coordinates based on highest number near toll booth location	Rocío Treviño Ulloa in Correo Real (2015) ^a
IH-10, 20 km west Ozone, Texas	30.69003	-101.42364	04-Nov-16	66/100m		Tracy (2018)
IH-10, 20 km west Sonora, Texas	30.62369	-100.84477	20-Oct-16	52/100m		Tracy (2018)
TX-137, northwest Sonora	30.75386	-101.20320	12-Oct-09	"numerous roadkills"	Location approximate	Journey North (2017) https://journeynorth.org/sightings/query_result.html?record_id=1445647488
IH-10, 16 km east Sheffield, Texas	30.7386	-101.65393	23-Oct-15	200		Journey North (2017) https://www.learner.org/jnorth/sightings/query_result.html?record_id=1255440284
Mexico 30 east of Celemania, Mexico	27.03486	-101.70802	31-Oct-15	"butterflies run over on Highway 30 Monclova-Cuatro Cienegas"	Location approximate	Prof. Daniel de Jesús Moreno González in Correo Real (2015) ^a



CorreoReal2015_MexicoRoadkillEnglishPart

^aEmbedded pdf for Correo Real (2015) with pertinent sections translated to English in red:

Table B.6. Monarch deaths related to mosquito adulticide ultra-low volume (ULV) truck spraying.^a

Location	Latitude	Longitude	Date	Approximate Number Dead Monarchs	Source	Comments
Central Park, New York	40.78224	-73.96722	20-Sep-99	“scores” [several times 20]	New York Daily News (1999)	Malathion; “St. Louis Encephalitis” vector spraying [later confirmed as West Nile Virus; Centers for Disease Control and Prevention 1999] Lethal levels of permethrin confirmed in monarch cadavers by Minnesota Dept. Agric. (St. Cloud Times 2000) ^b
Gaylord, Minnesota	44.55262	-94.22139	23-Aug-00	At least 320 counted “dozens”, including dragonflies	Journey North (2000)	
Grand Forks, North Dakota	47.92528	-97.03034	18-Aug-10		Grand Forks Herald (2010)	
Santa Catarina, Monterrey, Nuevo Leon, Mexico	25.67606	-100.44737	20-Oct-11	“hundreds”	www.Info7.mx (2011)	Dengue fever vector treatment

^aAll geocoordinates are approximate.

^bEmbedded pdf for St Cloud Times (2000):



St_Cloud_Times_Tues
day_7_November_2000

Table B.7. Latest fall months for mosquito adulticide ultra-low volume (ULV) spraying in eastern US counties.^a

Location	Latitude	Longitude	State	County	Latest ULV Spray Month	Date Latest ULV Spray	Sprayer Type	Source
Amelia Island	30.62763	-81.4534	Florida	Nassau	November	9-Nov-18	truck ULV	Amelia Island Mosquito Control District (2018)
Ames	42.02664	-93.6459	Iowa	Story	September	16-Sep	truck ULV in parks	Ames Tribune (2016)
Baldwin	38.596	-90.5452	Missouri	St Louis	September	late September	truck ULV	City of Baldwin (2018)
Bardstown	37.80608	-85.4676	Kentucky	Nelson	October	31-Oct-18	truck ULV	City of Bardstown (2018)
Bayside, Queens	40.75854	-73.7655	New York	Queens	September	19-Sep-18	truck ULV	New York City Health (2018)
Bel Air	39.53594	-76.3483	Maryland	Harford	September	early September	ground ULV	Bel Air (2018)
Bowling Green	36.9723	-86.4839	Kentucky	Warren	September	September through October	truck ULV	Warren County (2018)
Brandon	32.27146	-90.0001	Mississippi	Rankin	October	October	truck ULV	Brandon (2018)
Cameron	29.799	-93.3237	Louisiana	Cameron Parish	December	15-Dec-18	Ground and aerial ULV	Louisiana Mosquito Control Association (2016)
Chatham	31.99046	-81.1114	Georgia	Chatham	October	29-Oct-18	aerial and ground ULV	www.WTOC.com (2016)
Clayton	35.65112	-78.4659	North Carolina	Johnston	October	8-Oct-18	truck ULV	Clatyon (2018)
Colts Neck	40.2873	-74.1729	New Jersey	Monmouth	September	18-Sep-18	truck ULV	Monmouth County (2018)
Columbia	38.95059	-92.3251	Missouri	Boone	September	into September	truck ULV	City of Columbia (2018)

Table B.7 (cont.). Latest fall months for mosquito adulticide ultra-low volume (ULV) spraying in eastern US counties.^a

Location	Latitude	Longitude	State	County	Latest ULV Spray Month	Date Latest ULV Spray	Sprayer Type	Source
Conway	33.83695	-79.0446	South Carolina	Horry	November	through November	truck ULV	Horry County (2018)
Dallas	32.77661	-96.7968	Texas	Dallas	October	17-Oct	truck ULV based on surveillance	City of Dallas (2017)
Del Rio	29.37101	-100.896	Texas	Val Verde	October	18-Oct	truck ULV cover the city biweekly from late May to late October 2018	City of Del Rio (2018)
Delaware	39.15839	-75.5409	Delaware	Kent	October	mid October	ground and aerial ULV	Delaware Department of Natural Resources (2012)
Dublin	40.0989	-83.1125	Ohio	Franklin	September	18-Sep-18	truck ULV	City of Dublin (2018)
Fargo	46.86253	-96.7846	North Dakota	Cass	September	15-Sep-18	aerial ULV	Cass County Vector Control (2018)
Gainesville	38.79926	-77.6109	Virginia	Prince William	September	19-Sep-18	truck ULV	Prince William county (2018)
Geneva	41.88708	-88.3075	Illinois	Kane	August	15-Aug-18	truck ULV	Kane County Chronicle (2018)
Hattiesburg	31.32528	-89.2981	Mississippi	Forrest	October	October to	truck ULV	www.WDAM.co
Hiawatha	42.0498	-91.6682	Illinois	Linn	August	November 31-Aug-18	truck ULV	m (2018)
Hopkinsville	36.87017	-87.4905	Kentucky	Christian	October	2-Oct-18	truck ULV	Hiawatha (2018)
Ingersoll Township	43.51352	-84.2487	Michigan	Midland	September	17-Sep-18	truck ULV	Kentucky Department of Agriculture (2018)
Jackson	32.30517	-90.1942	Mississippi	Hinds	October	through October	truck ULV	www.ABC12.co m (2018)
								City of Jackson (2018)

Table B.7 (cont.). Latest fall months for mosquito adulticide ultra-low volume (ULV) spraying in eastern US counties.^a

Location	Latitude	Longitude	State	County	Latest ULV Spray Month	Date Latest ULV Spray end 30	Sprayer Type	Source
Jonesboro	33.5218	-84.3522	Georgia	Clayton	September	September	truck ULV	Clayton County (2018)
Lauderdale	44.99858	-93.2058	Minnesota	Ramsey	September	7-Sep-18	truck ULV routine truck and aerial ULV spraying occur throughout the city from late March to early November	Metropolitan Mosquito Control District (2018)
League City	29.50783	-95.0952	Texas	Galveston	November	16-Nov		League City (2016)
Louisville	38.24983	-85.732	Kentucky	Jefferson	October	12-Oct-18	truck ULV	www.LouisvilleKy.gov (2018)
Macon	32.81694	-83.6738	Georgia	Bibb	October	4-Oct-18	truck ULV	MaconBibb (2018)
McAllen	26.20048	-98.2266	Texas	Hidalgo	December	peak mosquito season October through December early September	truck ULV	www.KVEO.com (2018)
McDonough	33.44628	-84.1483	Georgia	Henry	September	2016	truck ULV	City of McDonough (2018)
Natchez	31.55192	-91.4064	Mississippi	Adams	October	end October "extend into November depending on the weather"	truck ULV	Natchez (2018)
New Orleans	29.95107	-90.0715	Louisiana	Orleans	November		aerial ULV	www.NOLA.com (2018)
Newton	41.05868	-74.7529	New Jersey	Sussex	October	10-Oct-18	truck ULV	Sussex County (2018)
Norman	35.21619	-97.4324	Oklahoma	Cleveland	October	10-Oct-18	truck ULV	www.newsok.com (2018)

Table B.7 (cont.). Latest fall months for mosquito adulticide ultra-low volume (ULV) spraying in eastern US counties.^a

Location	Latitude	Longitude	State	County	Latest ULV Spray Month	Date Latest ULV Spray	Sprayer Type	Source
Panama City	30.20495	-85.8424	Florida	Bay	October	3-Oct-18	truck ULV	Beach Mosquito Control District (2018)
Perkasie Borough	40.37205	-75.2927	Pennsyl- vania	Bucks	August	15-Aug-18	truck ULV	Pennsylvania West Nile Virus Control Program (2018)
Plantersville	33.55523	-79.2158	South Carolina	George- town	September	through September	truck and aerial ULV	Georgetown County (2010)
Riverton	43.01932	-108.385	Wyoming	Fremont	September September early	September early	truck ULV	City of Riverton (2018)
Sidney	41.14343	-102.978	Nebraska	Cheyenne	September	September	truck ULV	Sidney (2014)
Sioux Falls	43.53845	-96.7417	South Dakota	Min- nehaha	September	22-Sep-18	truck ULV	Sioux Falls Health Department (2018)
Thornton	39.87322	-104.971	Colorado	Adams	September	18-Sep-18	truck ULV	Vector Disease Control International (2018)
Tulsa	36.16108	-96.0048	Oklahoma	Tulsa	October	15-Oct-18	truck ULV	www.Newson6.c om (2018)

^aAll geocoordinates are general city locations.

Table B.8. Areas (mHa) and proportions of eastern monarch fall migratory flyway regions subject to mosquito ultra-low volume adulticide spraying and upper 75th percentile usage of both glyphosate and neonicotinoids by US counties.^a

State	Area States (mHa)	Percent Area States ^b	Area Mosquito ULV Spray Counties (mHa)	Percent Area Mosquito ULV Spray Counties ^c	Percent Area Mosquito ULV Spray Counties in States ^d	Area Glyphosate/Neonico-tinoid Counties (mHa)	Percent Area Glyphosate/Neonico-tinoid Counties ^c	Percent Area Glyphosate/Neonico-tinoid Counties in States ^d
<i>South Eastern Flyway Region (below 37°N)</i>								
Alabama	10.36	12.1%	2.42	6.8%	23.4%	0.42	8.5%	4.1%
Florida	7.38	8.6%	5.15	14.4%	69.8%	0.00	0.0%	0.0%
Georgia	15.24	17.8%	8.79	24.6%	57.6%	0.40	8.0%	2.6%
Kentucky	0.35	0.4%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
Louisiana	7.91	9.2%	4.38	12.2%	55.3%	1.28	25.8%	16.2%
Mississippi	4.46	5.2%	1.27	3.6%	28.5%	0.00	0.1%	0.1%
North Carolina	12.78	14.9%	3.12	8.7%	24.4%	1.02	20.6%	8.0%
South Carolina	8.01	9.4%	6.65	18.6%	83.0%	0.00	0.0%	0.0%
Tennessee	3.39	4.0%	0.14	0.4%	4.1%	0.00	0.0%	0.0%
Texas	12.61	14.7%	3.62	10.1%	28.7%	1.84	37.0%	14.6%
Virginia	3.08	3.6%	0.20	0.6%	6.4%	0.00	0.0%	0.0%
<i>Total/</i>								
Total Percent ^e	85.59	100.0%	35.74	100.0%	41.8% ^e	4.96	100.0%	5.8% ^e
<i>North Eastern Flyway (above 37°N)</i>								
Connecticut	1.29	2.6%	0.19	1.9%	15.1%	0.00	0.0%	0.0%
Delaware	0.53	1.1%	0.53	5.1%	100.0%	0.25	49.1%	47.2%
District of Columbia	0.02	0.0%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
Kentucky	0.67	1.4%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
Maine	8.44	17.3%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
Maryland	2.57	5.3%	1.15	11.0%	45.0%	0.00	0.0%	0.0%
Massachusetts	2.10	4.3%	1.36	13.0%	64.9%	0.00	0.0%	0.0%
New Hampshire	2.40	4.9%	0.19	1.8%	7.7%	0.00	0.0%	0.0%

Table B.8 (cont.). Areas (mHa) and proportions of eastern monarch fall migratory flyway regions subject to mosquito ultra-low volume adulticide spraying and upper 75th percentile usage of both glyphosate and neonicotinoids by US counties.^a

State	Area States (mHa)	Percent Area States ^b	Area Mosquito ULV Spray Counties (mHa)	Percent Area Mosquito ULV Spray Counties ^c	Percent Area Mosquito ULV Spray Counties in States ^d	Area Glyphosate/Neonico-tinoid Counties (mHa)	Percent Area Glyphosate/Neonico-tinoid Counties ^c	Percent Area Glyphosate/Neonico-tinoid Counties in States ^d
<i>North Eastern Flyway Region (below 37°N)</i>								
New Jersey	1.97	4.0%	1.97	18.8%	100.0%	0.00	0.0%	0.0%
New York	7.05	14.4%	0.45	4.3%	6.4%	0.00	0.0%	0.0%
Pennsylvania	7.45	15.2%	4.18	40.0%	56.2%	0.25	50.9%	3.4%
Rhode Island	0.28	0.6%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
Vermont	2.49	5.1%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
Virginia	7.30	14.9%	0.44	4.2%	6.0%	0.00	0.0%	0.0%
West Virginia	4.28	8.8%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
<i>Total/</i>								
Total Percent ^e	48.83	100.0%	10.47	100.0%	21.4% ^e	0.50	100.0%	1.0% ^e
<i>South Central Flyway (below 37°N)</i>								
Alabama	3.01	2.2%	0.58	4.3%	19.3%	0.16	1.1%	5.2%
Arkansas	13.77	10.1%	1.17	8.7%	8.5%	2.66	18.0%	19.3%
Colorado	0.02	0.0%	0.00	0.0%	0.0%	0.01	0.0%	31.5%
Illinois	0.00	0.0%	0.00	0.0%	0.0%	0.00	0.0%	6.2%
Kansas	0.01	0.0%	0.00	0.0%	0.0%	0.01	0.1%	87.5%
Kentucky	2.08	1.5%	0.92	6.8%	44.2%	0.50	3.4%	24.1%
Louisiana	4.17	3.1%	0.86	6.4%	20.7%	1.04	7.0%	24.9%
Mississippi	7.89	5.8%	0.90	6.7%	11.4%	1.97	13.3%	25.0%
Missouri	3.01	2.2%	0.14	1.0%	4.5%	0.79	5.3%	26.2%
New Mexico	20.58	15.1%	1.83	13.6%	8.9%	0.37	2.5%	1.8%

Table B.8 (cont.). Areas (mHa) and proportions of eastern monarch fall migratory flyway regions subject to mosquito ultra-low volume adulticide spraying and upper 75th percentile usage of both glyphosate and neonicotinoids by US counties.^a

State	Area States (mHa)	Percent Area States ^b	Area Mosquito ULV Spray Counties (mHa)	Percent Area Mosquito ULV Spray Counties ^c	Percent Area Mosquito ULV Spray Counties in States ^d	Area Glyphosate/Neonico-tinoid Counties (mHa)	Percent Area Glyphosate/Neonico-tinoid Counties ^c	Percent Area Glyphosate/Neonico-tinoid Counties in States ^d
Oklahoma	18.10	13.3%	1.36	10.1%	7.5%	1.06	7.2%	5.9%
Tennessee	7.52	5.5%	0.61	4.6%	8.2%	1.28	8.6%	17.0%
Texas	56.02	41.1%	5.10	37.8%	9.1%	4.96	33.5%	8.9%
<i>Total/</i>								
Total Percent ^e	136.19	100.0%	13.47	100.0%	9.9% ^e	14.80	100.0%	10.9% ^e
<i>North Central Flyway (above 37°N)</i>								
Colorado	17.26	6.0%	5.41	20.5%	31.3%	2.87	3.1%	16.6%
Illinois	15.01	5.2%	0.59	2.3%	4.0%	11.64	12.6%	77.6%
Indiana	9.44	3.3%	0.56	2.1%	6.0%	4.99	5.4%	52.8%
Iowa	14.58	5.1%	0.64	2.4%	4.4%	12.60	13.7%	86.4%
Kansas	21.30	7.4%	0.30	1.2%	1.4%	7.47	8.1%	35.1%
Kentucky	7.35	2.5%	1.85	7.0%	25.2%	0.48	0.5%	6.5%
Michigan	25.04	8.7%	0.68	2.6%	2.7%	2.34	2.5%	9.4%
Minnesota	22.53	7.8%	1.34	5.1%	6.0%	9.83	10.7%	43.6%
Missouri	15.05	5.2%	0.80	3.0%	5.3%	3.50	3.8%	23.2%
Montana	24.58	8.5%	0.00	0.0%	0.0%	1.79	1.9%	7.3%
Nebraska	20.02	6.9%	0.98	3.7%	4.9%	10.73	11.6%	53.6%
New York	6.57	2.3%	0.69	2.6%	10.5%	0.00	10.6%	0.0%
North Dakota	18.31	6.3%	1.76	6.7%	9.6%	9.75	4.0%	53.2%
Ohio	11.99	4.2%	2.94	11.2%	24.5%	3.72	3.1%	31.0%
Pennsylvania	4.32	1.5%	0.92	3.5%	21.2%	0.00	0.0%	0.0%
South Dakota	19.97	6.9%	1.38	5.2%	6.9%	8.49	9.2%	42.5%
West Virginia	2.00	0.7%	0.00	0.0%	0.0%	0.00	0.0%	0.0%
Wisconsin	16.93	5.9%	0.15	0.6%	0.9%	1.95	2.1%	11.5%

Table B.8 (cont.). Areas (mHa) and proportions of eastern monarch fall migratory flyway regions subject to mosquito ultra-low volume adulticide spraying and upper 75th percentile usage of both glyphosate and neonicotinoids by US counties.^a

State	Area States (mHa)	Percent Area States ^b	Area Mosquito ULV Spray Counties (mHa)	Percent Area Mosquito ULV Spray Counties ^c	Percent Area Mosquito ULV Spray Counties in States ^d	Area Glyphosate/Neonico-tinoid Counties (mHa)	Percent Area Glyphosate/Neonico-tinoid Counties ^c	Percent Area Glyphosate/Neonico-tinoid Counties in States ^d
Wyoming	16.45	5.7%	5.38	20.4%	32.7%	0.00	0.0%	0.0%
<i>Total/</i>								
Total Percent ^e	288.69	100.0%	26.37	100.0%	9.1% ^e	92.15	100.0%	31.9% ^e
<i>Coastal Funnel</i>								
Louisiana	7.88	36.2%	4.35	53.3%	55.2%	1.28	41.1%	16.3%
Mississippi	0.91	4.2%	0.20	2.4%	22.0%	0.00	0.1%	0.5%
Texas	13.00	59.7%	3.62	44.3%	27.9%	1.83	58.8%	14.1%
<i>Total/</i>								
Total Percent ^e	21.79	100.0%	8.18	100.0%	37.5% ^e	3.12	100.0%	14.3% ^e
<i>Central Funnel</i>								
Oklahoma	13.85	27.9%	1.36	26.3%	9.8%	0.54	20.5%	3.9%
Texas	35.75	72.1%	3.80	73.7%	10.6%	2.08	79.5%	5.8%
<i>Total/</i>								
Total Percent ^e	49.60	100.0%	5.16	100.0%	10.4% ^e	2.62	100.0%	5.3% ^e

^aSee Fig B.2 for monarch fall migratory flyway regions.

^bArea States/Total Area States

^cArea Mosquito ULV Spray Counties/Total Area Mosquito ULV Spray Counties

^dArea Mosquito ULV Spray Counties/Area States

^eTotal Area Mosquito ULV Spray Counties/Total Area States

Figures

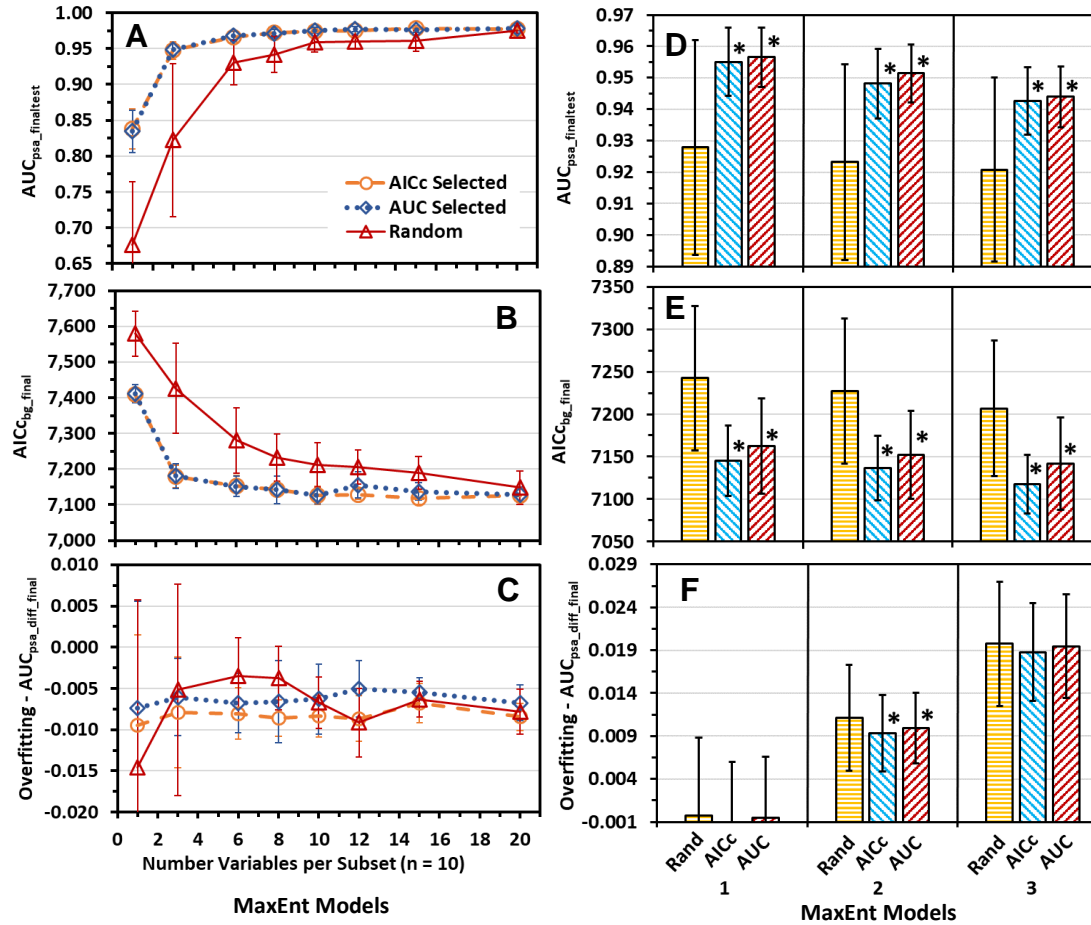


Fig. B.1 MaxEnt migration model evaluation statistics (mean \pm SD) of $AUC_{psa_finaltest}$ (A,D), $AICc_{bg_final}$ (B,E), and $AUC_{psa_diff_final}$ (overfitting; C,F) for models developed from (A-C) top ten variable subsets selected by AUC_{psa} or AICc using random subset feature selection (RSFSA) and ten random subsets out of 250 randomly generated six-variable subsets of various sizes derived from 80 variables (RSFSA Stage I); and (D-F) top 250 variable subsets out of 3,000 subsets per three training set replicates selected by AUC_{psa} or AICc using RSFSA and top 300 random generated six-variable subsets out of 3,000 subsets derived from 80 variables (RSFSA Stage II). Means for AUC_{psa} selected or AICc selected model statistics within a replicate with an asterisk are significantly more optimal (higher for $AUC_{psa_finaltest}$ and lower for $AICc_{bg_final}$ and $AUC_{psa_diff_final}$) from that of random selected models ($P < 0.05$; Welch t test with Holm correction, preceded by significant Welch ANOVA test, $P < 0.05$)

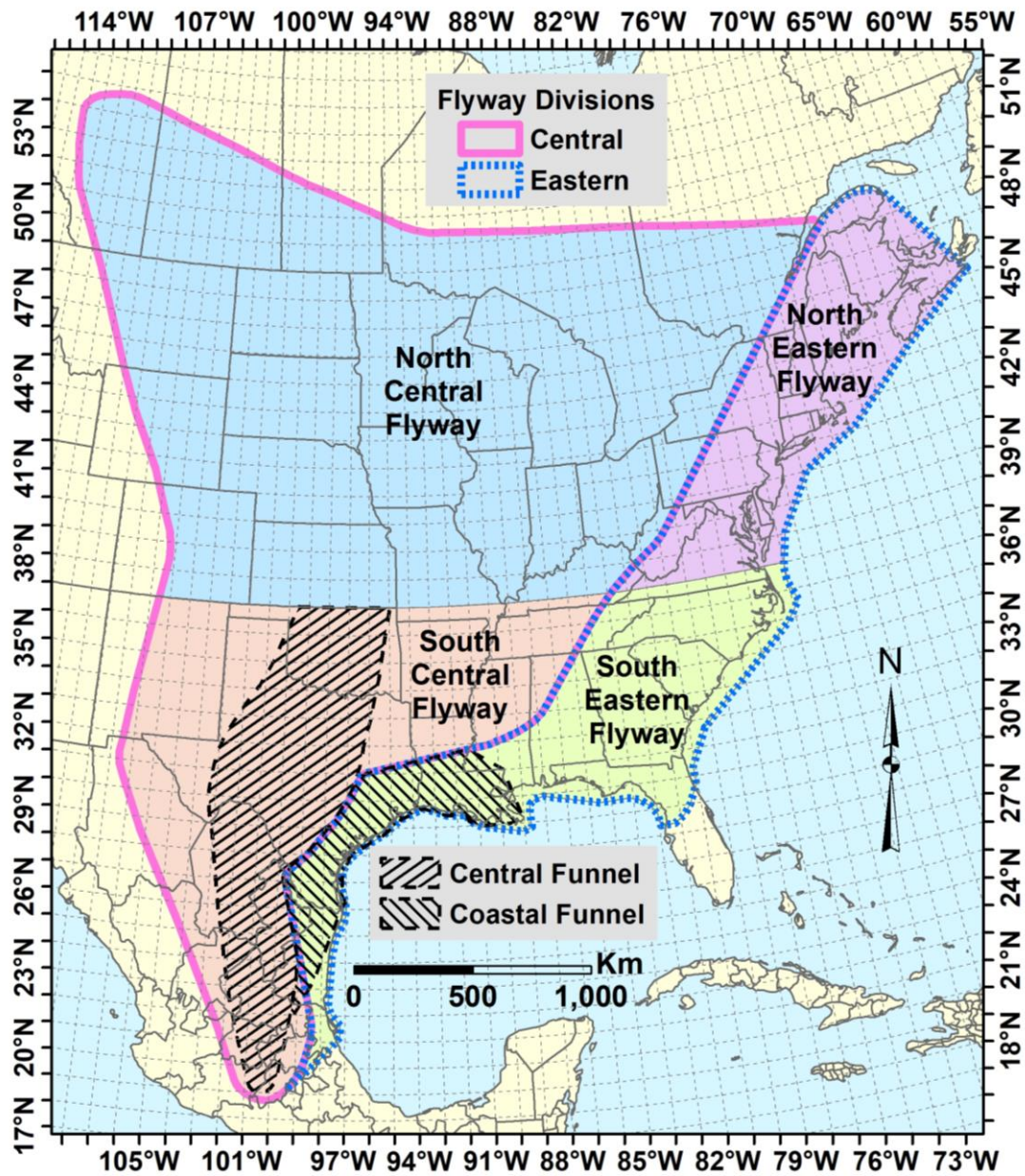


Fig. B.2 Regions of monarch fall migratory flyway divisions.

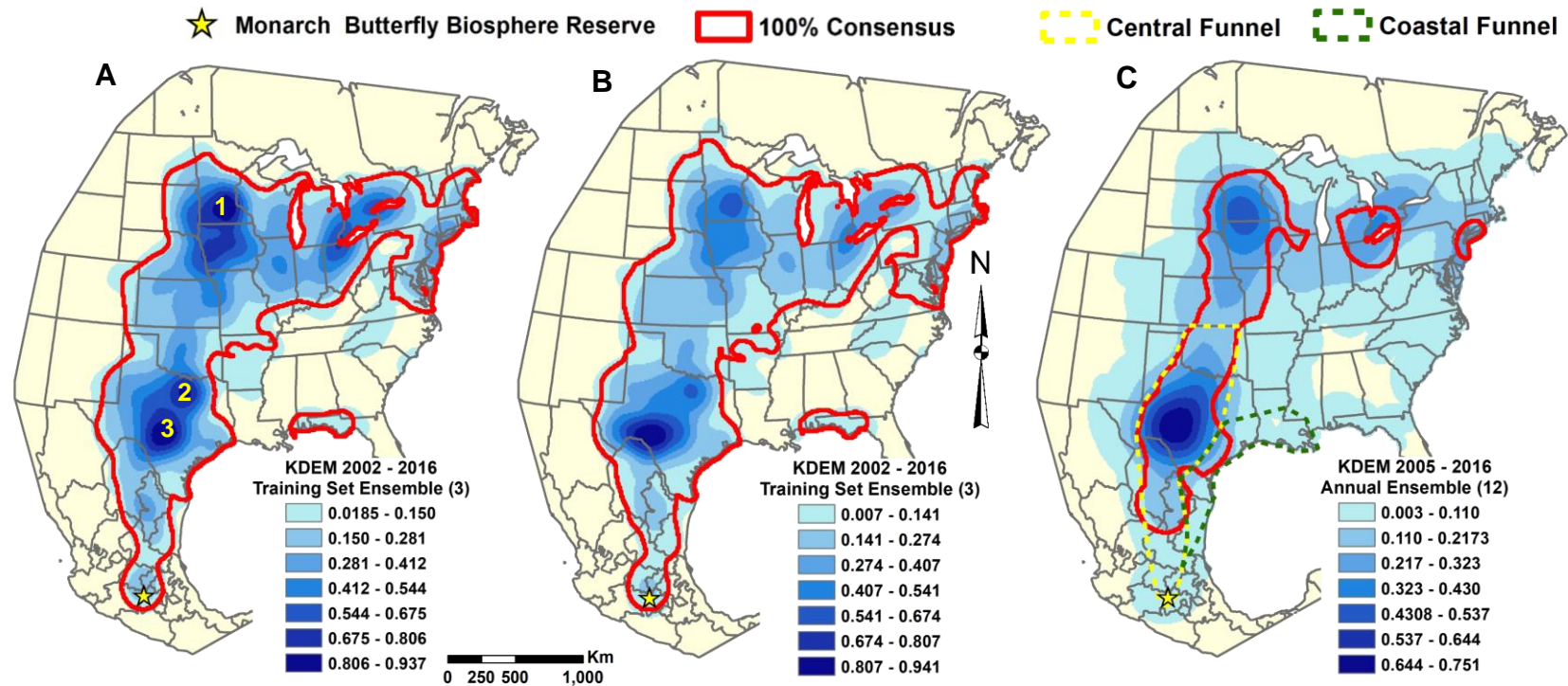


Fig. B.3 Monarch fall migration pathway minimum calibration of average consensus kernel density estimation model (KDEM) ensembles with 100% consensus boundaries of training set models ($n = 3$) for 2002-2016 without (A) and with (B) reduction of human observer bias using the monarch roost human population density index (Fig. 2B), and (C) annual models ($n = 12$) from 2005–2016, including the Central Funnel and Coastal Funnel core migration pathways (see Fig. 5 for individual annual minimum consensus models). Metropolitan areas of Minneapolis/St Paul (1), Dallas/Fort Worth (2), and Austin/San Antonio (3) are identified in (A) (see Table B.4 for shapefiles).

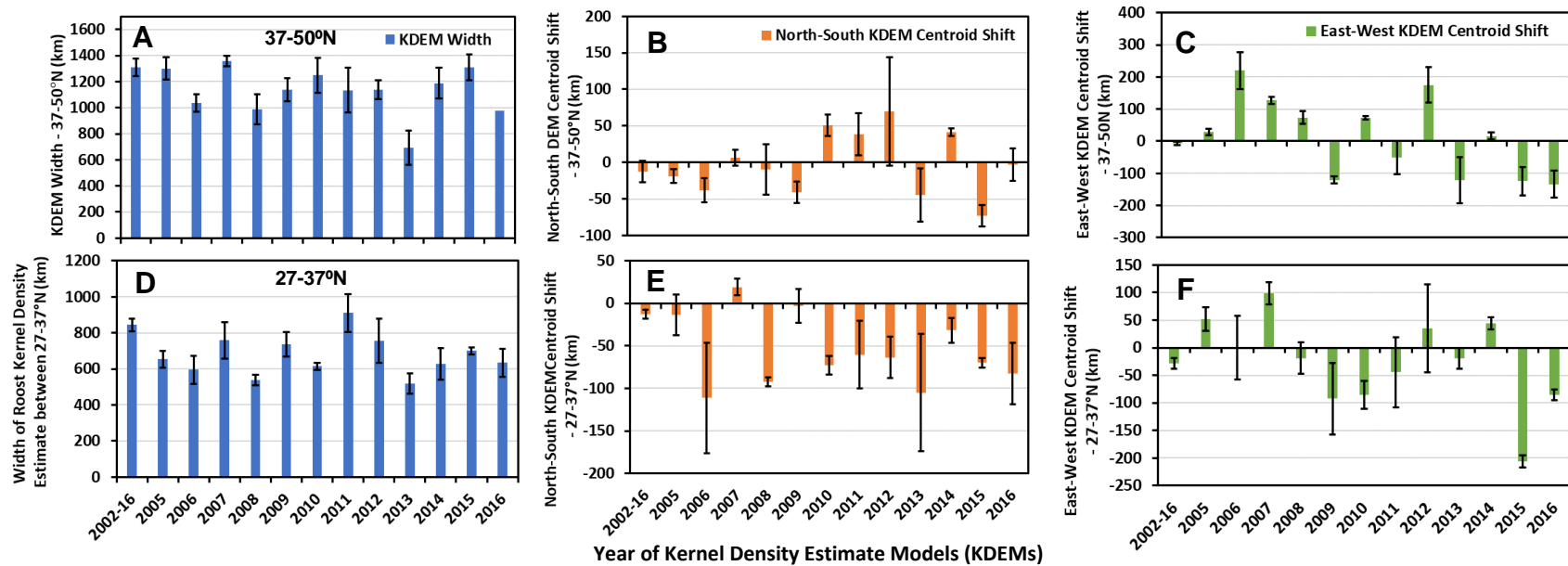


Fig. B.4 Monarch migration pathway characteristics for the Central Flyway from training set ensembles of kernel density estimation models (KDEMs) (n = 3) for 2002–2016 and annually from 2005 to 2016 for (A-C) northern area from 37 to 50°N and (D-F) southern area from 27 to 37°N. Includes (A,D) the average KDEM width and the shift of the KDEM centroid north or south (B,E) or east to west (C,F) compared to the 2002–2016 minimum frequency consensus KDEM.3

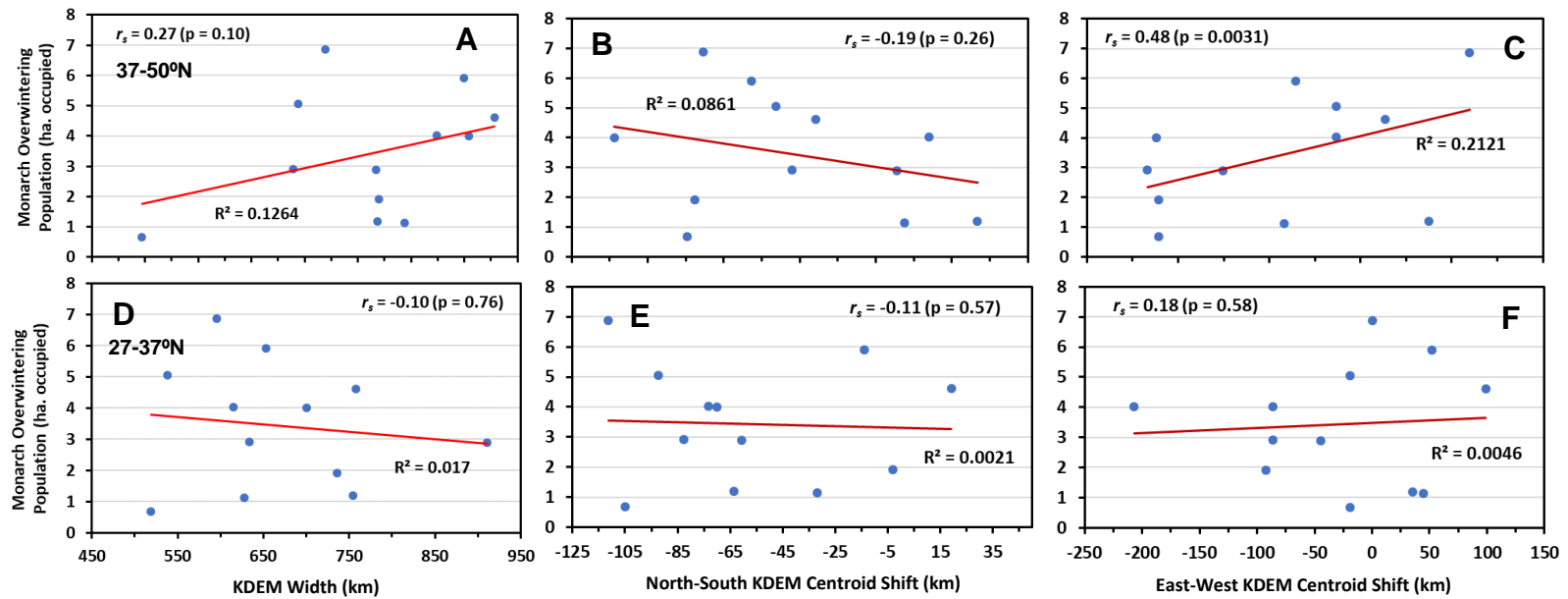


Fig. B.5 Spearman rank correlation (r_s) between annual 2005 to 2016 monarch overwintering population areas in Mexico and monarch migration pathway characteristics in the Central Flyway from training set ensembles of kernel density estimation models (KDEMs) ($n = 3$): (A-C) northern area from 37 to 50°N and (D-F) southern area from 27 to 37°N. Includes (A,D) the average KDEM width and the shift of KDEM centroid north or south (B,E) or east to west (C,F) compared to 2002–2016 minimum frequency consensus KDEM (see Fig. B.4 for original dimensional characteristics).

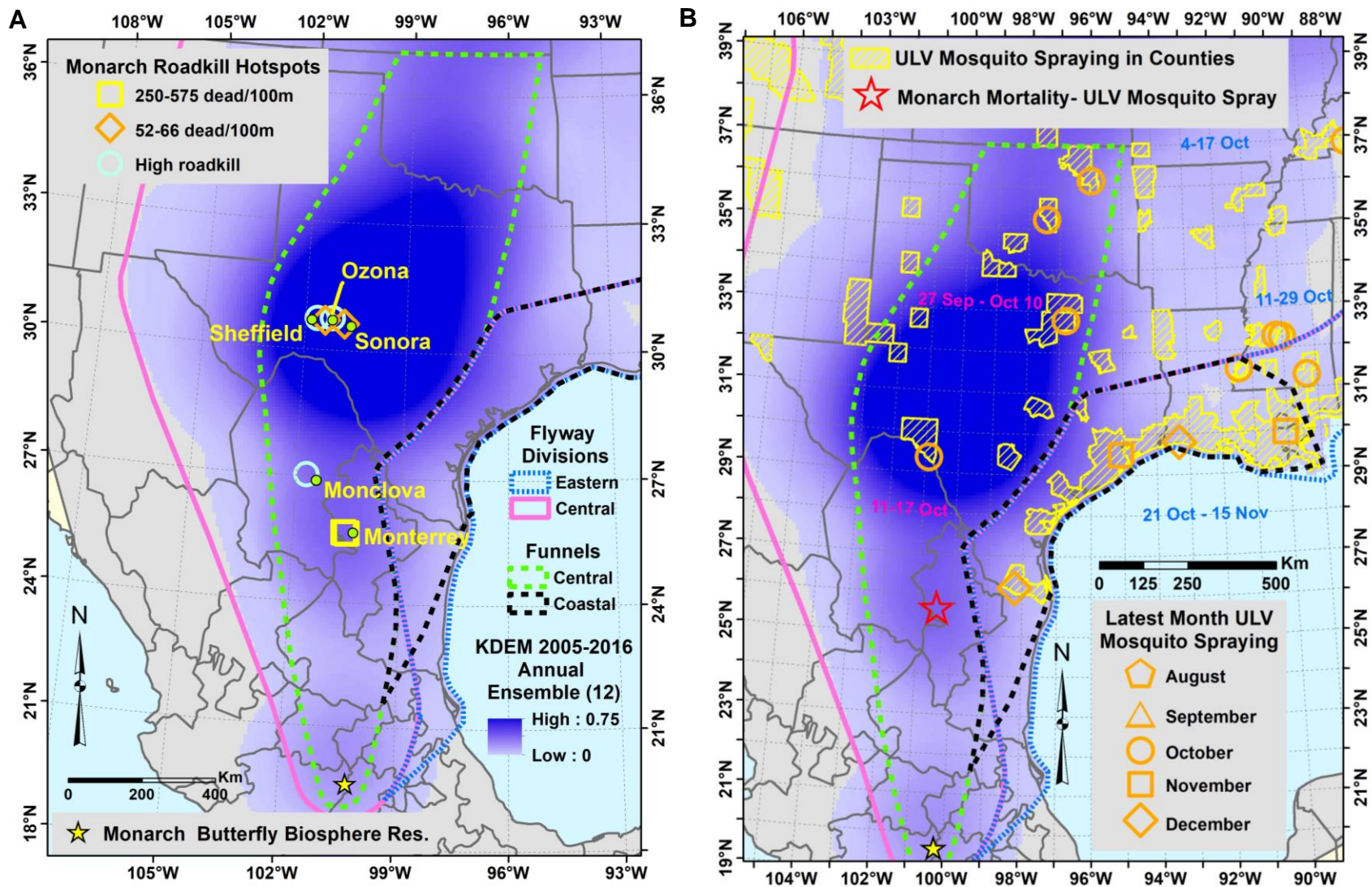


Fig. B.6. Monarch fall migration pathway Central and Coastal funnels and annual kernel density estimation models (KDEMs) for 2005-2016 ($n = 12$, blue shading; see Fig. 4 for individual annual KDEMs) and potential anthropogenic hazards: (A) monarch roadkill hotspots; (B) US counties with 75th percentile level uses of glyphosate herbicide and neonicotinoid insecticides (clothianidin, imidacloprid, and thiamethoxam) in 2014.

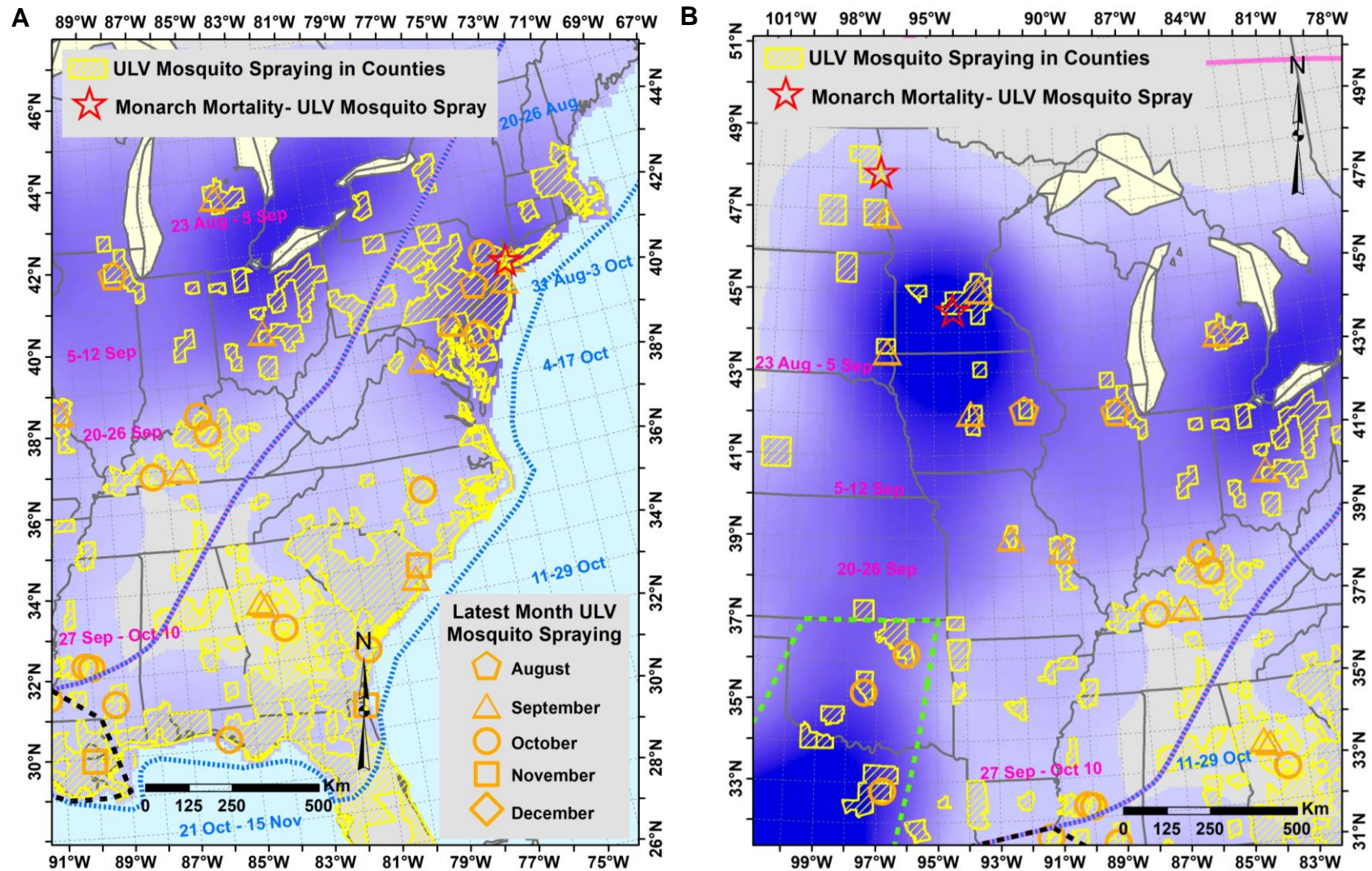


Fig. B.7 Monarch fall migration pathway (A) eastern Eastern Flyway and (B) northern Central Flyway with annual kernel density estimation models (KDEMs) for 2005-2016 ($n = 12$, blue shading; see Fig. 4 for individual annual KDEMs) and monarch mortality from late season mosquito adulticide ultra-low volume (ULV) spray treatments, months for latest ULV spraying, approximate dates for peak migration along the Central Flyway (pink text) and Eastern Flyway (blue text) (Calvert and Wagner 1999; Howard and Davis 2009), and US counties with ULV spraying.

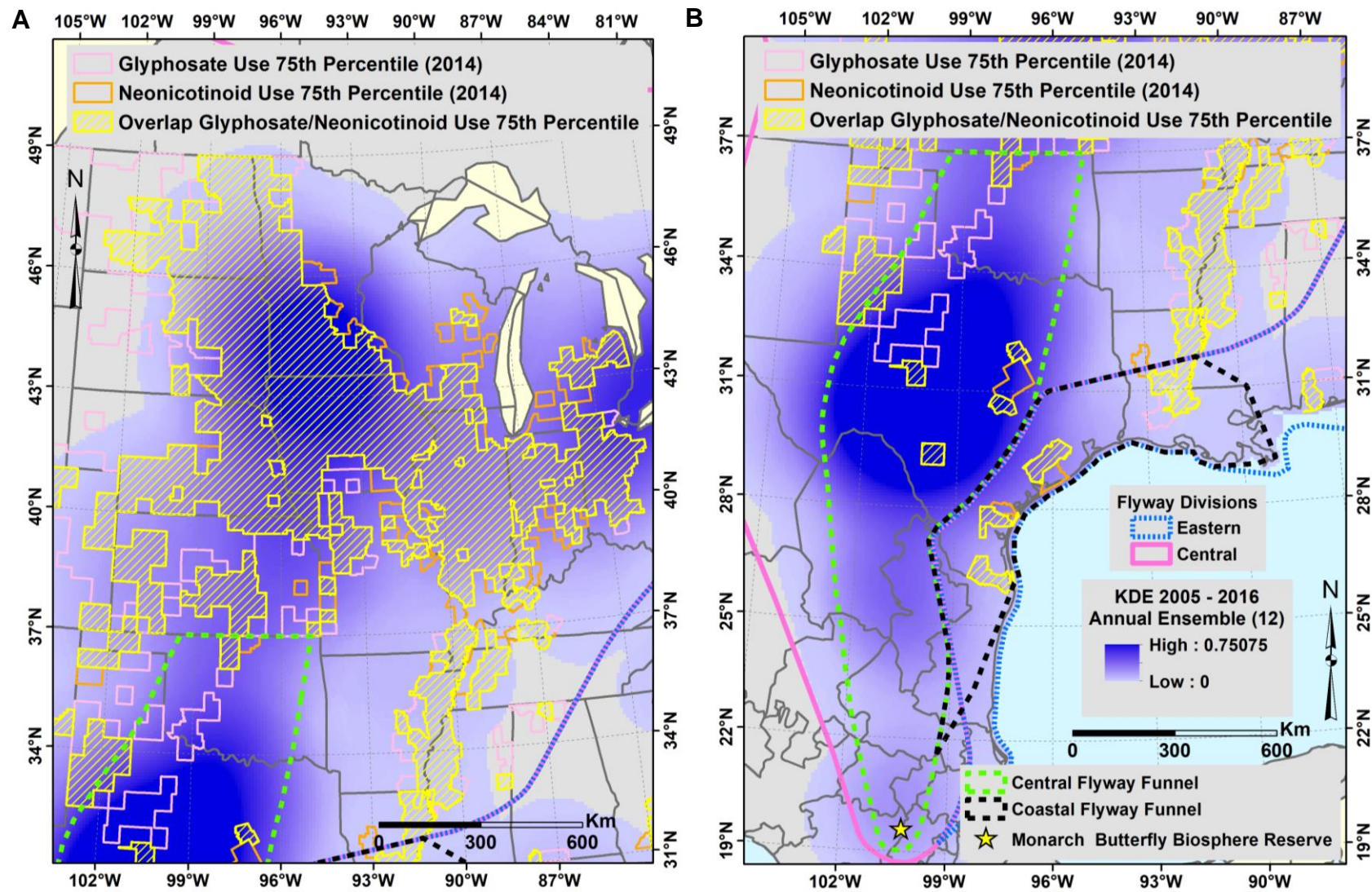


Fig. B.8. Monarch fall migration pathway (A) northern Central Flyway and (B) Central and Coastal funnels with annual kernel density estimation models (KDEs) for 2005-2016 ($n = 12$, blue shading; see Fig. 4 for individual annual KDEs) and US counties with 75th percentile level uses of glyphosate herbicide and neonicotinoid insecticides (clothianidin, imidacloprid, and thiamethoxam) in 2014.

APPENDIX C:
MANUSCRIPT SECTION II;
SPATIAL RISK ASSESSMENT OF EASTERN MONARCH BUTTERFLY
ROAD MORTALITY DURING AUTUMN MIGRATION WITHIN THE
SOUTHERN CORRIDOR

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Abstract

Road mortality may contribute to the population decline of eastern monarch butterflies (*Danaus plexippus* L.). We estimated autumn monarch roadkill rates within the primary Oklahoma to Mexico southern migration corridor (i.e., Central Funnel). Dead monarchs were surveyed along Texas roadsides during four weeks of autumn migration in 2016 and 2017. Roadkill averaged 3.4 monarchs per 100 m transect, reaching 66 per 100 m in a roadkill hotspot in southwestern Texas. Extrapolations of Central Funnel roadkill based on survey data and road types were 3.6 and 1.1 million in 2016 and 2017, respectively. Spatial distribution of roadkill across the Central Funnel was projected from Texas survey data using 30 m resolution MaxEnt niche models. Highest roadkill probability was linked to arid climate and low human population density. The latter variables may not be directly related to roadkill, but instead represent indirect correlates of increased densities of monarchs where the migration corridor narrows southwards. The higher roadkill projected in southwest Texas and Mexico by MaxEnt models agrees with previously reported monarch roadkill hotspots. MaxEnt-based 2016-2017 projections for annual roadkill rates throughout the Central Funnel averaged 2.1 million. This figure is similar to the result by simple extrapolation, and represents about 3% of the overwintering monarch population for these years. Mitigation at roadkill hotspots in the Central Funnel could reduce monarch roadkill mortality during migration and contribute towards conservation efforts for the monarch butterfly.

Keywords: *Danaus plexippus*; Migratory threats; MaxEnt roadkill niche models; Insect roadkill hotspots

1. Introduction

Wildlife-vehicle collision is the most widely acknowledged impact of roads on wildlife, and can contribute to the decline of species of conservation concern (Tok et al. 2011, Visintin et al. 2016, Bennet 2017), including globally declining pollinator insect species (Baxter-Gilbert et al. 2015). Roadkill can result in high mortality and lower abundance for species with large area requirements, pronounced migratory movements, small population sizes, and slow reproduction rates (Seiler and Helldin 2006, Fahrig and Rytwinski 2009). Wildlife-vehicle collisions are often spatially and temporally aggregated and substantial annual and inter-annual variation has been associated with environmental factors and traffic volume (Seiler and Helldin 2006, Shilling and Waetjen 2015). This tendency for high spatio-temporal variability in roadkill can be difficult to interpret based solely on the mapping of field survey data. Accordingly, there is a trend to use predictive spatial models to account for the variability in investigating the impacts of roads on wildlife mortality (Bennet 2017). Typical roadkill niche models use a combination of environmental and anthropogenic variables and are often restricted to small areas (Visintin et al. 2016). Species distribution modeling has previously been used to project roadkill risk of mammals (Grilo et al. 2009, Roger and Ramp 2009,

Visintin et al. 2016, 2017) and owls (Gomes et al. 2009). Spatial models for insect roadkill have not yet been developed.

Only five out of 215 roadkill studies (2%) between 2011 and 2015 were specifically focused on invertebrates (Bennet 2017). Despite this taxonomic bias in the literature, roadkill may be a substantial threat to certain insect populations. Baxter-Gilbert et al. (2015) projected that hundreds of billions of pollinating insects are lost annually to roadkill across North America. Although relatively few studies exist on butterfly road mortality, researchers have suggested that butterflies are one of the most common insect orders killed by vehicles (McKenna et al. 2001, Rao and Girish 2007). Despite the high numbers of road-killed butterflies, the risk of Lepidopteran roadkill has been estimated as low to moderate (Baxter-Gilbert et al. 2015, Muñoz et al. 2015). Several studies have estimated butterfly roadkill numbers and examined contributing factors (Munguira and Thomas 1992, McKenna et al. 2001, Ries et al. 2001, Rao and Girish 2007, Skórka et al. 2013), but most of these studies concentrate on local, relatively sedentary butterfly populations and their utilization of roadside habitats rather than migratory butterflies. Migratory danaine butterflies (Nymphalidae: Danainae) may be especially susceptible to roadkill during migration (Her 2008, Taiwan Environmental Protection Administration [EPA] 2010, Santhosh and Basavarajappa 2014), including the monarch butterfly (*Danaus plexippus* L.) in the United States (McKenna et al. 2001) and Mexico (Correo Real 2015).

Significant population decline of the eastern migratory monarch butterfly has been observed at the overwintering sites in Central Mexico during the past two decades (Vidal and Rendón-Salinas 2014, Thogmartin et al. 2017). Consequently, the persistence of the migratory phenomenon of the eastern population may be endangered (Brower et al. 2012). The long-distance migration of monarchs is unique among butterflies. Mortality during the autumn migration (often referred to as fall migration) has been suggested as a contributing factor to the decline (Badgett and Davis 2015, Ries et al. 2015, Inamine et al. 2016, Agrawal and Inamine 2018). Road mortality may significantly affect monarch survival during migration, especially where monarchs become highly concentrated as the migration narrows in Texas and northern Mexico (Badgett and Davis 2015). Only McKenna et al. (2001) have previously evaluated monarch butterfly roadkill. They reported monarchs as the second-most killed butterfly species during six weeks of autumn in Illinois. They estimated that more than 500,000 monarchs were killed statewide along interstate highways during one week in early September 1999 (McKenna et al. 2001). There are several unpublished citizen-science reports of locally high monarch roadkill occurrence in West Texas and northern Mexico during the autumn migration, with observed roadkill reaching 5.7 monarchs per meter near Monterrey, Mexico (Correo Real 2015, Journey North 2017). Incidences of high monarch road mortality in northern Mexico have led to the placement of road signs along portions of highways in to reduce speed in the presence of monarchs (Vanguardia 2016).

Monarch roadkill has yet to be quantified in relation to an overall population estimate. Seiler and Helldin (2006) point out that any sustained mortality factor, such as roadkill, can be especially damaging for species that are either approaching or are in an annual population

decline, such as the monarch. The extent of monarch roadkill needs to be assessed to estimate its potential contribution to the population decline and support conservation planning. Our goal was to develop MaxEnt niche models for monarch road mortality during the autumn migration within the main migration pathway in Texas. The MaxEnt algorithm has been employed before to spatially investigate vehicle-animal collisions of birds and mammals (Ha and Shilling 2017). The roadkill models were also projected throughout the Central Funnel, which is the identified main southern autumn migratory pathway within the Central Flyway for monarchs from Oklahoma to Mexico (Tracy 2018, Tracy et al. 2018a). This study includes the first analysis of monarch roadkill data outside of Illinois, and the first development of a spatial roadkill model for an insect. Our specific objectives were to (1) conduct monarch roadkill field surveys within the Central Funnel in Texas, (2) develop MaxEnt niche models for roadkill within the Texas survey area, and project these models throughout the Central Funnel, (3) estimate monarch roadkill numbers within the survey area and the Central Funnel using both simple field survey-based and model-based extrapolation techniques, and (4) discuss the results in the context of monarch conservation and potential applications to other species of conservation concern.

2. Methods

2.1 Study species

The monarch autumn migration is uniquely accomplished by one generation. Adults begin migrating in late August to September from the summer breeding grounds, traveling to overwintering grounds in Central Mexico (Brower 1995, Calvert and Wagner 1999). Most migrants usually reach Oklahoma and North Texas in late September or early October (Calvert and Wagner 1999, Monarch Watch 2018a) and arrive at the overwintering grounds in November (Brower et al. 2006). There are two main migration routes, the Central Flyway and the Eastern or Coastal Flyway (Calvert and Wagner 1999, Howard and Davis 2009). The Central Flyway through the Great Plains is the most heavily traveled route (Howard and Davis 2009), which narrows into the Central Funnel from Oklahoma southwards (Tracy 2018, Tracy et al. 2018a).

Autumn migrants fly during the day and stop at night and during inclement weather to nectar and roost in trees and shrubs (Brower 1996). These roosts may comprise a few individuals to several thousand individuals and may last one to several days (Davis and Garland 2004, Howard and Davis 2009). In the morning, roosting monarchs either resume migration or search for nectar. These behaviors are influenced by wind patterns or lipid levels (Brower 1996, Davis and Garland 2004). During unfavorable southerly winds, monarchs may roost for several days (Schmidt-Koenig 1985). Migrating monarchs are observed to nectar in a variety of locations, including in right-of-ways (Brower et al. 2006), where they may be vulnerable to vehicle collisions. Brower et al. (2006) suggested that monarchs shift their behavior upon reaching Texas and spend more time nectaring to accumulate lipids for the winter and re-migration in the spring. Migrating monarchs regularly fly at high altitudes, around 300-500 m (Gibo and Pallett 1979, Gibo 1981, 1986), but may fly close to the ground, especially when facing headwinds or during overcast weather (Gibo 1986, Brower 1996), exposing them to road

mortality. Citizen science observations also include reports of low flying fall migrating monarchs over roadways (Correo Real 2015).

2.2 Monarch roadkill surveys and simple roadkill extrapolation

Monarch roadkill field surveys were conducted during the main autumn migration through the Central Funnel in Texas (Fig. 1). Four four-day surveys were conducted in each of the autumns of 2016 and 2017, between 10th October to 4th November and 3-27 October, respectively. The survey area was divided into four north to south sections, with surveys timed to generally occur after the dates of average peak migration (Journey North 2017, Monarch Watch 2018a) to allow time for the accumulation of road-killed monarchs. Observed monarch roadkill densities most likely represent accumulations of dead migrating monarchs over a period of one or two days to a few weeks during the main migration pulse through an area (Munguira and Thomas 1992). Surveyed road types included (1) highways, (2) primary roads, and (3) secondary roads. Each survey location comprised at least a single 100 m by one m transect along the grassy edge of one side of the roadway. To assess if the side of the road surveyed influenced the number of dead monarchs, additional transects were surveyed across multiple edges of single and divided-lane highways at some sites. Transects were located using a handheld GPS device (accuracy up to ± 3 m). Roadkill transects were spaced according to travel and survey time constraints at about 30 to 100 km intervals along the primarily east to west pre-planned survey routes, with additional surveys in 2016 in the southwestern portion of the study area where high monarch roadkill was found (Fig. 1).

All dead monarchs or parts of monarchs were collected to estimate the total number and sex ratio of dead monarchs along transects. A similar spring monarch roadkill survey was conducted in Texas during April to May of 2017 (Fig. A.1; for details, see Appendix A, section 1.1). The boundary of the background evaluation extent for our roadkill study was defined by a 10 km buffer around a convex hull polygon formed using un-thinned 2016 to 2017 monarch roadkill survey data (Fig. 1). We extrapolated the mean roadkill counts for the three road types over the background evaluation extent and Central Funnel in a manner similar to that of McKenna et al. (2001). Roadkill rates in areas of locally high monarch roadkill (hotspots) can vary greatly between years and highly differ from other areas. Consequently, roadkill rates in hotspots can bias roadkill rates in larger areas if they are disproportionately represented in the sampling. Therefore, in some extrapolations, hotspot roadkill rates were considered separately for each year from non-hotspot locations. This separation allowed us to understand the importance of the roadkill hotspots within a year, and compare the hotspot differences between years. We also made roadkill extrapolations including hotspot roadkill rates with non-hotspot data and thinning the hotspot data in 2016 to be in proportion to the sample effort in non-hotspot locations (for details, see Appendix A, section 2.1).

2.3 Environmental variables

Thirty environmental variables were initially screened for use in the roadkill modeling (Table A.1, Fig. A.2). These variables were selected for their value in previous roadkill niche models

and for their use in characterizing the environment of the study area. The variables consisted of nine topographic indices (including four stream indices), eight land cover indices, six road indices, three human population indices, and four climatic indices. All indices were either calculated at 30.8 m spatial resolution or resampled with bilinear interpolation to the 30.8 m resolution, to match the resolution of the base layer of one arc second Shuttle Radar Topography Mission (SRTM) digital elevation model (DEM) data obtained from USGS Earth Explorer (<https://earthexplorer.usgs.gov/>). The high spatial resolution of 30.8 m facilitated modeling of roadkill along individual surveyed roadways over a broad area (see more details in Appendix A, section 7).

2.4 Monarch roadkill models

Preliminary MaxEnt model runs indicated that there was not enough data from 2017 to obtain good accuracy statistics for both single year models. Consequently, we combined 2016 and 2017 roadkill presence data, which were randomly spatially thinned to 2 km to reduce spatial autocorrelation. Ten thousand background points were randomly generated within the road mask evaluation area. We calculated background/presence versions of the area under the curve statistic (AUC_{bgp}) and true skill statistic (TSS_{bgp}) using R software (R Core Team 2018) and the PresenceAbsence package (Freeman and Moisen 2008). In the same manner, we calculated a presence/absence version of AUC (AUC_{pa}) and TSS (TSS_{pa}) using transects with no observed monarch roadkill as absence data, although we acknowledge roadkill may have occurred in these absence locations as well. We adjusted the MaxEnt beta regularization value to two and used only quadratic and hinge features to reduce model complexity and overfitting for improving model generalization (Jiménez-Valverde et al. 2008, Warren and Seifert 2011, Tracy et al. 2018b).

Roadway rasters served as a mask for analysis of environmental variables. The original set of 30 environmental variables was decreased to 20 variables. We dropped nine variables exhibiting zero or negative testing gain of AUC_{bgp} from a MaxEnt threefold jackknife run (Table A.1, Fig. A.3). We also dropped traffic volume because data were not readily available for Mexico. Traffic volume was utilized in preliminary niche models for the Texas background evaluation extent. Preliminary runs indicated that no substantial gain in model performance was achieved with more than 10 of the 20 variables, and that employing fewer variables (three) substantially increased undesired high spatial variability in roadkill among models. Consequently, final MaxEnt roadkill models were developed from ten random sets of ten of the 20 variables to represent model variability due to variable selection. The absolute Spearman rank correlation of variables within random variable sets was limited to less than 0.7 using the multiple randomized sequential forward selection procedure within the random subset feature selection algorithm (RSFSA) of Tracy et al. (2018b). The final MaxEnt models were calibrated to binary presence/absence format using a threshold of maximum TSS_{pa} (Liu et al. 2013) and combined using frequency consensus to form a feature subset ensemble. We also created presence/absence niche models for monarch roadkill using linear and quadratic binomial logistic regression with the R Glmnet package (Friedman et al. 2010) for the same ten random sets of ten of 20 variables. These presence/absence models produced lower AUC_{bgp} values and

similar AUC_{pa} values compared to MaxEnt models, and these models were not investigated further (results not shown).

3. Results

3.1 Monarch roadkill survey and simple roadkill extrapolation

We surveyed 16.1 km of roadsides (161 100 m transects), 8.8 km in 2016 and 7.3 km in 2017. We found 581 dead monarchs in 59 locations (102 absence locations) for an average of 3.4 monarchs per transect. We found 546 dead monarchs in 2016 and 35 in 2017 (for raw survey count data, see Appendix A, section 3.1). Of the 546 monarchs in 2016, 499 (91%) were located along or near IH-10 between Sonora and Sheffield (23 of 95 transects) (Fig. 2). This area was defined as a single large 2016 hotspot area extending 95 km along Interstate Highway (IH) 10 from Sonora to 24 km east of the Pecos River. This hotspot included a portion of Texas state highway (SH) 163 extending from 7.6 km south of Ozona to 5 km north of Ozona and a 2 km section of SH-137 extending west from the junction with SH-163. The hotspot was bounded along IH-10 in the west and east by counts of 15 and 10 roadkill per 100 m, and included counts of 21 and 6 roadkill per 100 m on SH-137 and SH-163, respectively. About 93% (466/499) of the dead monarchs in the 2016 hotspot occurred in 14 transects ranging from ten to 66 per 100 m (Fig. A.4). A total of 257 monarchs were sexed in 2016-2017, of which ca. 38% were female (n=98). The portion of females was 41% in 2016 (132 males, 91 females) and 21% in 2017 (27 males, 7 females). After the 2 km spatial thinning for spatial modeling, the field survey data consisted of 151 transects (53 presence and 98 absence) and 249 individual dead monarchs. Only two road-killed male monarchs were found among 54 transects in our spring 2017 roadkill survey (Fig. A.1).

Southern edges of roadsides contained 43.8% (\pm 36% SD, n = 13) of the roadkill found in the northernmost edge for both single and divided-lane roads. This relationship was used to estimate roadkill across all two to four edges per roadway when extrapolating roadkill per km per year (for details, see Appendix A, section 5). Estimated roadkill per km ranged from 6.15 to 645 monarchs per km depending on year, road type, and location in relation to the hotspot area (Table 1). In 2016, mean estimated roadkill per km along highways was significantly higher within the Sonora-Sheffield hotspot (645.38; SD = 446.92; n = 15) compared to outside the hotspot (12.64; SD = 22.30; n = 22) (P = 0.00008; Welch t-test). Between years, the estimated roadkill per km generally varied widely among road types, and there was no evidence of any hotspot in 2017 (Table 1). Estimates of roadkill within the hotspot area in 2016 and 2017 ranged from about 74,000 to 5,000, representing about 10% and 1 % of the entire mortality within the Texas background evaluation extent. Using the field survey data from the whole area, including the randomly thinned hotspot data, the estimated roadkill for the Central Funnel in 2016 rose to over 3.5 million monarch butterflies (Table 1). The estimate for 2017 was 1.1 million road-killed monarchs. Combining the data from both of the years yielded an annual estimate of 2.1 million road-killed monarchs. When the hotspot data were separated from the survey data, total estimated annual roadkill rates in 2016 and 2017 were about 1.6 million and 1.0 million monarchs, respectively. In 2016, about 47%-49% of the roadkill within the Central

Funnel was projected to occur within the Texas background evaluation extent, of which 5% was projected to occur in the Sonora-Sheffield hotspot area. In 2017, only about 0.3% of the roadkill within the Central Funnel was projected within the hotspot area, and the percent roadkill in the background area ranged from 31% when hotspot data were analyzed separately to 48% when data from the hotspot area were included (Table 1, Appendix A, section 2).

3.2 Monarch roadkill model

The feature subset ensemble of ten MaxEnt roadkill niche models provided excellent discrimination of roadkill presence locations from background locations within the background evaluation extent, with AUC_{bgp} values ranging from 0.82 to 0.88 (0.86 ± 0.02 , mean \pm SD). The models provided marginally poor discrimination of roadkill presences from absences observed in the field, with AUC_{pa} values ranging from 0.60 to 0.67 (0.64 ± 0.02 , mean \pm SD). Among the top eight variables with the highest permutation importance in the MaxEnt models (Table 2) were three human population density indices and two climatic indices. Other top ranked variables included elevation (m), road density over a three km radius ($km/\sim 28 km^2$), and percent cover of artificial surfaces within a 500 m radius. Traffic volume (annual average daily traffic, AADT) ranked third in permutation importance in preliminary models but was not available for Mexico, precluding its use in our final models.

The MaxEnt response curves for population density indices all indicated that lower human population densities had higher association with monarch roadkill (Fig. 3 A, E). The climatic indices indicated that roadkill was associated with more arid climates (Fig. 3 B, C). Lower road densities and lower percent cover of artificial surface were also associated with higher monarch roadkill (Fig. 4 F, G). The response curve for traffic volume, which was not used in the final models, indicated that the highest roadkill was associated with lower traffic volume. The traffic volume response curve was very similar to that of percent cover of artificial surface (Fig. 3 G, H), with which it was moderately correlated ($r_s = 0.58$).

The proportion of MaxEnt models projecting monarch roadkill generally increased from northeast to southwest within both the background evaluation extent and Central Funnel (Fig. 4; see Appendix Section 5 for embedded zipped shapefile of MaxEnt consensus model). The highest number of models projected roadkill within much of southwest Texas and Mexico in the Central Funnel. None of the MaxEnt models projected monarch roadkill over most of the northeastern Central Funnel, including the Dallas/Fort Worth metroplex and most of eastern Oklahoma.

Roadkill extrapolations for each of the ten individual MaxEnt models were made across each year and both years combined. Extrapolations were based upon roadkill rates per km for presence-only transects (no zero roadkill data) of each road type that were multiplied by the MaxEnt model predicted lengths of roadkill presence for the road type. Roadkill rate calculations for extrapolations included data from hotspot transects that were thinned for 2016 as done for simple extrapolations (for details, see Appendix A, sections 2.1, 5). Extrapolating roadkill rates across the Central Funnel, we estimated averages of 3.0 ± 0.7 and 1.1 ± 0.3

million (mean \pm SD) road-killed monarchs for 2016 and 2017, respectively (Tables 1, A.2). Combining roadkill data across both years for the Central Funnel yielded annual roadkill rates of 2.1 ± 0.5 million. The mean projected percentage of road-killed monarchs in the Central Funnel that occurred within the Texas background evaluation extent ranged from 67% to 68% in 2016 and 2017 (Tables 1, A.4).

4. Discussion

4.1 Monarch roadkill survey

In our 2016 roadkill survey (but not 2017), we found a Sonora-Sheffield, Texas, monarch roadkill hotspot that corresponds to the only two previous citizen-science reports of monarch roadkill hotspots in the US (Fig. 2). It is unclear why hotspots have repeatedly occurred in this area. The hotspot location may be partly related to higher densities of migrating monarchs in more southern areas of the Central Funnel. In addition, local stochastic weather events probably influence the occurrence of roadkill hotspots, such as unfavorable winds that may induce lower monarch flight patterns or extended roosting and nectaring behavior close to the ground in the vicinity of roadways. More research is needed to evaluate how frequently roadkill hotspots occur in this region. It is possible that one or both years represent an outlier, and that hotspots may occur in additional areas.

The variation in roadkill rates observed between the two years of our survey was consistent with other roadkill studies (Seiler and Helldin 2006). Our overall roadkill rates varied from 6 to 646 dead monarchs per km depending on year, road type, and location (Table 1). This range falls within previous reports for monarchs of from 1.3 to 11.9 butterflies per km per week in Illinois (McKenna et al. 2001) to a very high number of 115 road-killed monarchs within a 20 m stretch along toll highway 40D southwest of Monterrey, Mexico in October 2015 (Correo Real 2015; see Appendix A, section 8 for data). Reported roadkill rates for other butterflies have ranged from 0.45 to 80 per km per day in North America, Asia, and Europe (Rao and Girish 2007, De la Puente et al. 2008, Yamada et al. 2010, Skórka et al. 2013, Baxter-Gilbert et al. 2015).

The observed sex ratios of roadkill monarchs were also consistent with previous studies. About 38% of the dead monarchs in our autumn field surveys were females, ranging from 21% in 2017 to 41% in 2016. These figures generally match two separate citizen science observations on the percentage of female monarch roadkill in Mexico of 27% and 36% in October 2015 (Correo Real 2015). They also are within the range reported from the other roadkill study (McKenna et al. 2001), as well as studies of sex ratios during migration (Borland et al. 2004) or on the overwintering grounds (Steffy 2015). Davis and Rendón-Salinas (2010) found a decreasing trend ($\sim 10\%$) in percent female monarchs at the Mexican overwintering sites from 1976–2008, which they suggested could reflect female biased mortality due to the protozoan parasite *Ophryocystis elektroscirrha*.

While our estimates of monarch roadkill are likely conservative, carcass persistence is not likely to be a major source of bias. However, we did not evaluate persistence in our study, which could vary based on various factors, such as region, time of year, and weather conditions. Munguira and Thomas (1992) placed butterfly specimens on roadsides and found that only one of their 50 specimens disappeared during two weeks (daily loss rate of 0.15%). Several factors contribute to the conservative nature of our estimates, including the difficulty in detection of dead monarchs, especially in taller vegetation, shredding of dead monarchs by roadside mowing, and a portion of the monarchs remaining attached to the colliding vehicles (McKenna et al. 2001, Seiler and Helldin 2006). However, several studies indicate that road killed butterflies are mostly blown to the roadside edge, with individuals rarely trapped in car grills (Munguira and Thomas 1992, Ries et al. 2001). The overall agreement of our roadkill results with previous studies in terms of the range of roadkill rate variability and sex ratios supports the reliability of the data for extrapolating monarch road mortality according to road types and spatial modeling.

4.2 Monarch roadkill models

4.2.1. Roadkill projections

Most of the MaxEnt models projected monarch roadkill from the southwestern portion of the Central Funnel from West Texas to Mexico (Fig. 4). Our MaxEnt consensus projection agrees with all seven of the previously known citizen science reports of monarch roadkill hotspots in North America (Fig. 4), including two hotspots in West Texas (Journey North 2017) and five hotspots in northern Mexico (Correo Real, 2015; Rogelio Carrerra, Universidad Autonoma de Nuevo Leon, Nuevo Leon, Mexico, personal communication).

Our annual MaxEnt based roadkill estimates for monarch mortality throughout the Central Funnel were 3.0 to 1.1 million for 2016 and 2017, respectively. These MaxEnt roadkill estimates were similar to those based on simple roadkill extrapolation by road type when hotspot data were included (3.6 and 1.1 million, respectively). Most of the roadkill projected by MaxEnt models outside of the study area occurred in Mexico, indicating that more MaxEnt models are projecting roadkill along the sparser road network within the Central Funnel over northern and central Mexico than in the northern parts of the funnel (Fig. 4). As the autumn migration pathway narrows in the South, migrating monarchs become more concentrated in the Central Funnel. This higher concentration may contribute to higher roadkill densities in the southern parts of the Central Funnel in Mexico, where most previous reports of monarch roadkill hotspots originate (Fig. 4). Although our models project some increased southward mortality risk, additional data are needed to assess the extent of this risk in Mexico. The extrapolations including the hotspot data with other roadkill data and projecting higher annual roadkill of up to 3.6 million in the Central Funnel should be more realistic considering the occurrence of multiple roadkill hotspots in Mexico of higher density than seen in Texas. The MaxEnt models project little to no roadkill in the northeastern part of the funnel area, including the Dallas/Fort Worth area and eastern Oklahoma. However, some roadkill likely does occur

in this area, but possibly at a lower rate than in the more southern areas of the Central Funnel. Further research and field surveys are needed to verify roadkill rates outside of our survey area in the southern and northern parts of the Central Funnel. Additional data may allow effective use of roadkill density models, rather than presence only MaxEnt models used in this study. Roadkill density models can better reveal regional roadkill patterns useful in refining projections, especially for northern Mexico where roadkill could be much higher than in Texas. Roadkill field surveys from other parts of the autumn migration pathways could improve estimates for the total impact of roadkill on the monarch population.

4.2.2. *Factors affecting roadkill*

MaxEnt projections of monarch roadkill within the Central Funnel were generally associated with more arid climate and less densely populated areas (Fig. 3). These conditions generally describe those for the seven previous monarch roadkill hotspots reported from Texas and Mexico, with the possible exception of the roadkill hotspot in the vicinity of Monterrey, Mexico (Fig. 4). This could be related to a variety of factors. For example, autumn migrating monarchs have been observed to spend additional time flying lower to the ground during the afternoon in desert areas, perhaps to seek shelter from the heat or find nectar (Journey North 2018). Monarchs may need to spend more time searching for nectar in arid environments, although this has not been evaluated. Finally, the increased roadkill rates may simply reflect the increased number of monarchs in more southern areas of the Central Funnel. This southern locality factor cannot be associated with most of the predictors, with the exception of latitude, but it may have the highest influence. Local climate, weather patterns, and geography affect monarch movement and behavior, and they all are likely important contributors to road mortality. Wind patterns (direction, duration, and speed) may especially be more important than anthropogenic factors, but short-term weather events could not be incorporated in the models. Occurrence of these weather events is also highly variable and difficult to predict.

Traffic volume has been noted as one of the most important variables in previous roadkill studies (Bennet 2017). Traffic volume ranked high in importance in our preliminary roadkill models, but we found that model accuracy (AUC) was not significantly affected by its removal. Other variables that were correlated with traffic volume likely compensated for its absence, including human population density, artificial surface cover, distance to urban areas (km to population $\geq 300/\text{km}^2$), and road density. In our models including traffic volume, the highest roadkill was associated with fairly low AADT values, similar to the study by McKenna et al. (2001). In general, higher roadkill has been associated with higher traffic volume due to increased probability of vehicle collisions (Seiler and Helldin 2006, Skórka et al. 2013). Samways (1994) suggested that roads with high traffic volume serve as corridors for high butterfly mortality. In our study, low traffic volume, along with related anthropogenic variables, is correlated with locations of monarch roadkill hotspots, but may not be directly related to the roadkill mortality. As the human population grows, traffic volume should increase, including in the lower traffic volume monarch roadkill hotspot areas, likely leading to higher roadkill rates (Bennet 2017).

4.2.3 Spatial and temporal variation

Over 70,000 monarch roadkill were estimated in the Sonora to Sheffield, Texas hotspot in 2016, compared to about 5,000 in 2017. This illustrates the high spatio-temporal variability of roadkill and the potential contribution of hotspots to the monarch road mortality. The timing of our field surveys relative to the peak migration in those years may have varied among transect locations and years, which could have influenced our results. If peak migration occurred after our field surveys in one or both years, we may have underestimated roadkill numbers. For example, the autumn migration in 2017 was later than usual due to unusually hot summer (Agrawal and Inamine 2018). Few other studies have identified roadkill hotspots for butterflies (but see Samways 1994, Her 2008). Monarch roadkill hotspots may vary from year to year and may be difficult to locate or may not occur in some years.

We observed much higher monarch road mortality during autumn migration than in the spring (Fig. A.1), indicating that seasonality is a factor contributing to monarch roadkill rates. However, the monarch spring migration is more spatio-temporally dispersed making comparisons difficult. Temporal variability can occur between years, within the migration phase, or even within a day. For example, monarch behavior, such as flying low in the mornings (McKenna et al. 2001), probably affects the risk of roadkill at different times of day. Temporal patterns affecting monarch roadkill should be investigated further. Other sampling methods may be effective in detecting additional roadkill hotspots, such as adaptive cluster sampling, which was developed for inventorying scattered and clustered phenomena (Thompson 1990). More frequent samples in a given location can also be critical for detecting roadkill hotspots (Santos et al. 2015).

4.3 Implications and impacts in relation to monarch conservation

4.3.1 Autumn migration mortality

Butterfly roadkill is a density independent mortality factor (Rodewald and Gehrt 2014) with the incidence of roadkill being subject to high variability. Consequently, roadkill percentage of the migrating population is very unpredictable in any given year. We estimated a conservative annual percentage of migrating monarchs subject to road mortality in the Central Funnel by dividing a roadkill estimate by the sum of overwintering populations and roadkill estimate (overwintering cohort). The size of the overwintering populations is calculated by multiplying the hectares of roosting monarchs at the Mexican overwintering sites from Rendón-Salinas et al. (2018) by the value of 21.1 million monarchs per ha suggested by Thogmartin et al. (2017). Based on these amounts, the estimated annual roadkill rates from our combined years of 2016-2017 MaxEnt models in the Central Funnel represent an average of 2.8% (2.1 million) of the overwintering cohort. In 2016, roadkill within the Central Funnel may have represented about 4.0% of the overwintering cohort (Table 1). The corresponding roadkill for 2017 ranges from 1.5% to 1.8% of the overwintering cohort. In contrast, the projection of 500,000 road-killed monarchs by McKenna et al. (2001) in Illinois during one week in 1999 represents only 0.26%

of the overwintering cohort that year (191 million, 9.05 ha), indicating potentially lower roadkill rates for areas north of the Central Funnel in some years.

The actual monarch population decline within a given year can be much higher than the differences in overwintering cohort sizes from year to year, since it includes the unknown quantities of butterflies recruited, or not recruited due to habitat loss, during spring and summer breeding. Mortality at the Mexican overwintering sites due to occasional winter storms can produce very high mortality, estimated at around 75% in 2002 (Brower et al. 2004) and greater than 40% in 2016 (Brower et al. 2017). Average monarch overwintering mortality is around $36\% \pm 21\%$ ($n = 7$; range 4-55%) based on data from Ries et al. (2015; see their Fig. 24.7). Lowered recruitment due to loss of milkweed habitat has been identified as the primary factor in monarch population declines (Pleasants 2017, Thogmartin et al. 2017), with losses of milkweed resources in Iowa estimated at 76% from 1999-2014 (Pleasants et al. 2017). Other studies place more importance on mortality during the autumn migration in the decline of monarchs (Badgett and Davis 2015, Inamine et al. 2016, Agrawal and Inamine 2018). Consequently, it is complex to evaluate the relative importance of roadkill to other factors in the monarch population decline, but it is probably lower compared to the factors of milkweed habitat loss and overwintering mortality. Northward expansion of the monarch summer breeding range is anticipated with climate change (Batalden et al. 2007, Lemoine 2015), making the southward autumn migration route even longer, increasing both exposure to traffic and associated road mortality (Badgett and Davis 2015). Monarch roadkill during autumn migration should be further evaluated in the context of other mortality factors along the migration path (Baxter-Gilbert 2015).

Brower et al. (2012) fitted an exponential decline curve to the estimated monarch overwintering populations (ha) in Mexico from 1995 (winter 1994-1995) to 2011 ($P = 0.015$, $R^2 = 0.336$). Inclusion of seven additional years of data through 2018 (Vidal and Rendón-Salinas et al. 2014, Monarch Watch 2018b) strengthens fit of an exponential curve ($y = ae^{bx}$ Fig. 5) ($P = 0.0009$; adjusted $R^2 = 0.486$; ZunZun.com 2018). A concave exponential curve represents the most serious form of species population decline, indicating constant proportional negative pressure on the population (Di Fonzo et al. 2013). A standard geometric population growth curve, fit to the modeled exponential curve, reveals an average -7.21% annual population decline over the last 23 years associated with an overall 82% population reduction (for details, see Appendix A, section 8). In comparison, a fitted exponential curve for 1994-2011, as used by Brower et al. (2012), yielded a slightly lower annual decline rate of 6.46%. The updated results indicate that the decline rate may have increased or at least has not slowed down. Continuance of the 7.21% rate of decline would result in an average of 0.24 ha of overwintering monarchs occurring in 29 years (by 2046-2047), greatly increasing the chance of extirpation of eastern migrating monarchs by an extreme winter storm mortality event as the overwintering population crosses below the 0.25 ha quasi-extinction threshold (Semmens et al. 2016). A total reversal of the 7.21% annual monarch decline, coupled with an annual 7.21% population increase (net change 14.4%), would be needed to restore the size of the overwintering population to current conservation goal of six hectares (Thogmartin et al. 2017) over the next 15 years. A 0.5% annual reduction in migrating monarch mortality through roadkill mitigation could

significantly contribute to a reversal in the long-term 7.2% annual exponential decline in monarch populations (Fig. 5).

4.3.2 Roadkill mitigation

While some major factors in the monarch decline are difficult to reduce, such as overwintering mortality, the opportunity for reducing road mortality is possible through roadkill mitigation (see Rytwinski et al. [2016] for a review on mammal roadkill mitigation). The potential ability to locate re-occurring monarch roadkill hotspots could facilitate more effective mitigation. Scattered and unpredictable monarch roadkill would be more difficult to mitigate. A variety of roadkill mitigation measures have been implemented for danaine migratory butterflies. In Taiwan, a four-meter high net was placed along a 400 m section of bridge on National Freeway 3 to successfully induce spring migrating purple crow butterflies (*Euploea* spp.) to fly over and above the traffic, reducing on site roadkill from around 2.5% to 0.5%. In addition, an outer traffic lane was closed when more than 500 butterflies per minute were crossing (Her 2008, Taiwan EPA 2010). In response to heavy autumn migratory monarch roadkill observed in Coahuila state of Mexico, traffic signs were posted in 2015 limiting the maximum speed to 60 km per hour (37 mph) in the presence of monarchs (Miranda 2015). Police have been observed slowing traffic in Nuevo Leon state in Mexico to reduce monarch mortality along a highway south of Monterrey (Dr. Orley R. Taylor, personal communication). Additional research is needed to test and assess the effectiveness of these types of butterfly roadkill mitigation strategies for monarch roadkill hotspots in west Texas and Mexico.

6. Conclusions

Our study represents a novel approach for projecting roadkill of a migratory insect through ecological niche modeling. Annual monarch roadkill rates during autumn migration varied substantially. We found close agreement between two methods of estimating monarch roadkill rates, simple extrapolation by road type and MaxEnt roadkill model projections. We project about 1.0 to 3.6 million road-killed monarchs per year during autumn migration over the Central Funnel, which could represent 2-4% of the Mexican monarch overwintering population. MaxEnt model roadkill projections also aligned with several previously known monarch roadkill hotspots, which suggests MaxEnt models could be used to identify additional monarch roadkill within the Central Funnel. Roadkill rates may differ throughout the Central Funnel compared to those observed in the Texas survey area, and should be further investigated. Monarch road mortality should also be investigated along the Coastal Funnel, especially from Texas to Mexico. We recommend more detailed investigation into the spatial and temporal variability in monarch road mortality in the Central Funnel, including how local and short-term weather events, especially related to wind, influence monarch roadkill hotspots. With the new information on monarch road mortality in the Central Funnel, conservation efforts could be implemented to mitigate mortality at monarch roadkill hotspots. Reducing roadkill rate over the Central Funnel for the eastern monarch population is as an important step towards reversing the continuing decline of this iconic butterfly.

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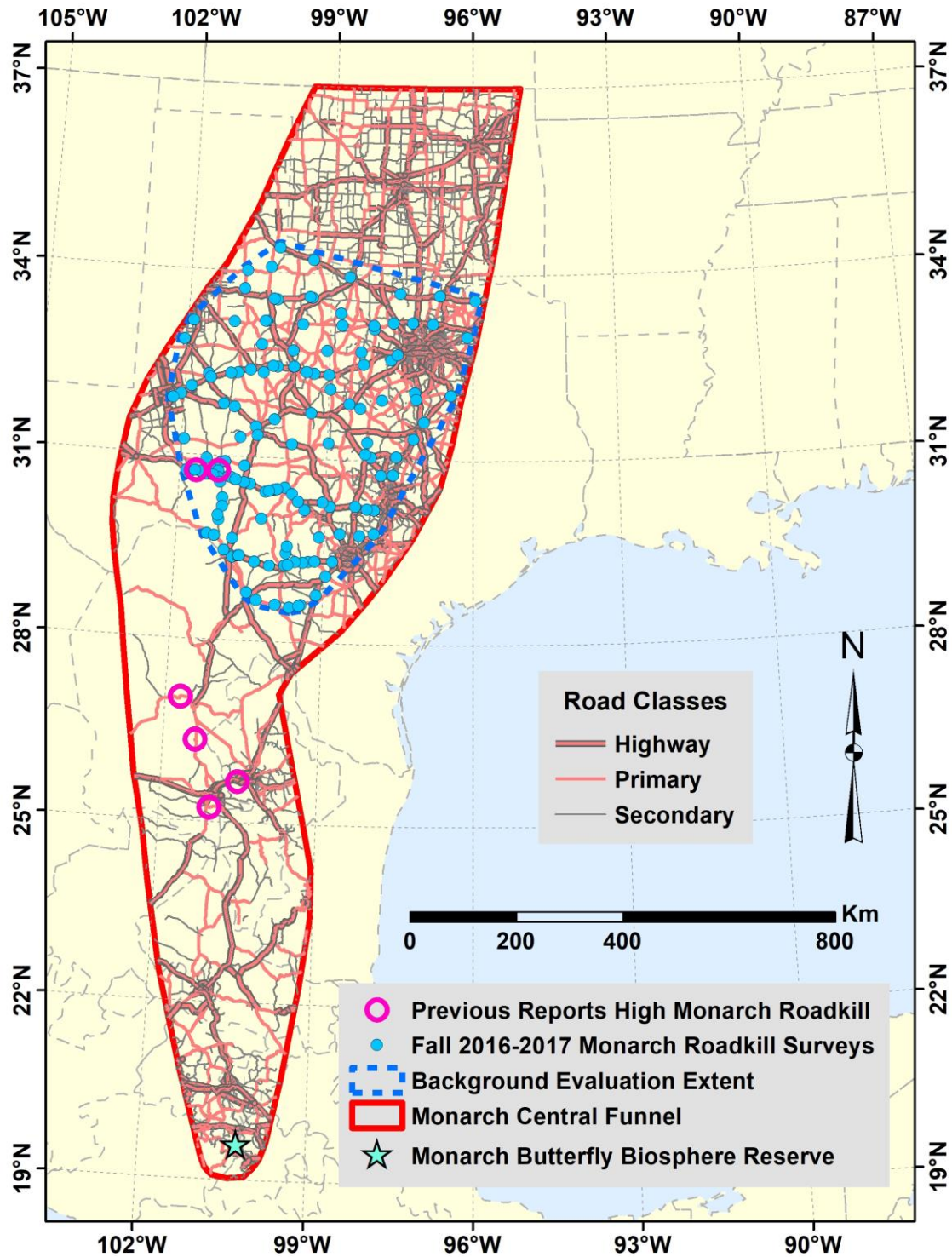


Figure 1. Monarch roadkill survey 100 m transects for autumn 2016 and 2017 along three major road classes within the monarch Central Funnel in Texas. [2 Columns; Color Figure Online]

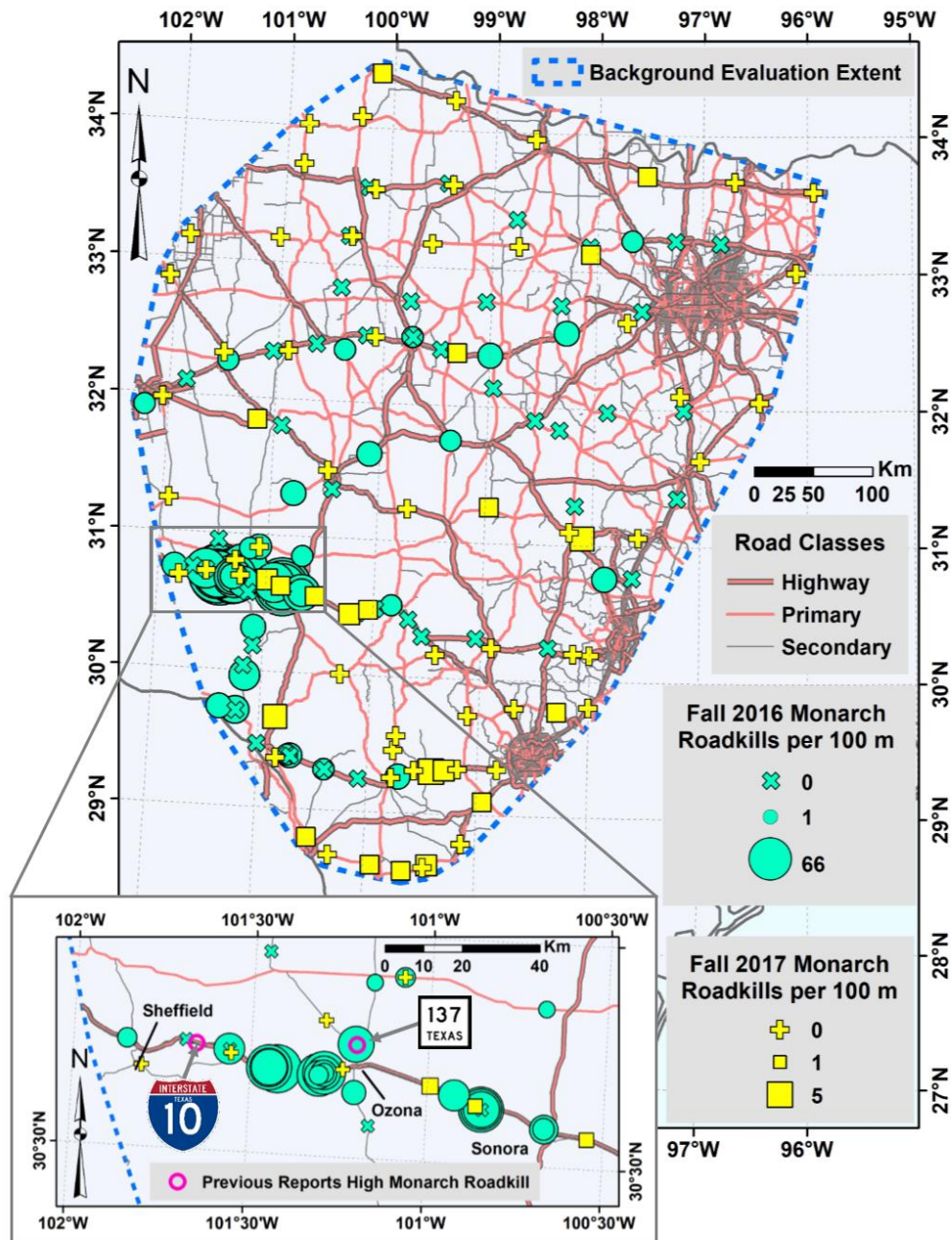


Figure 2. Monarch roadkill autumn 2016 and 2017 survey results for 100 m transects along major road classes within the background evaluation extent in the monarch Central Funnel in Texas, including previously reported locations of high monarch roadkill. [2 Columns; Color Figure Online]

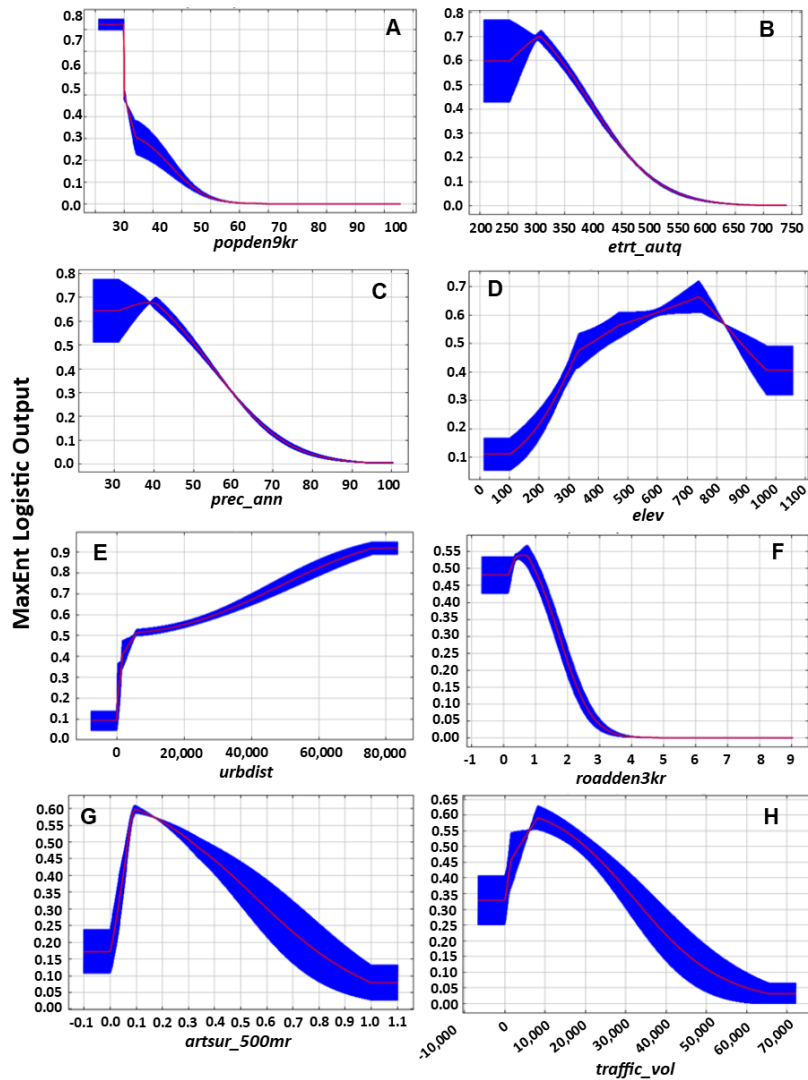


Figure 3. MaxEnt variable response curves (logistic output probability of presence vs. variable) representative of the final ten models (A–G) and for a 30-variable model (H): (A) popden9kr, (B) etrt_autq, (C) prec_ann, (D) elev, (E) urbdist, (F) roadden3kr, (G) artsur_500mr, (H) traffic_vol (traffic volume for 2015) (see Table 2 for abbreviations and permutation importance). [2 Columns; Color Figure Online]

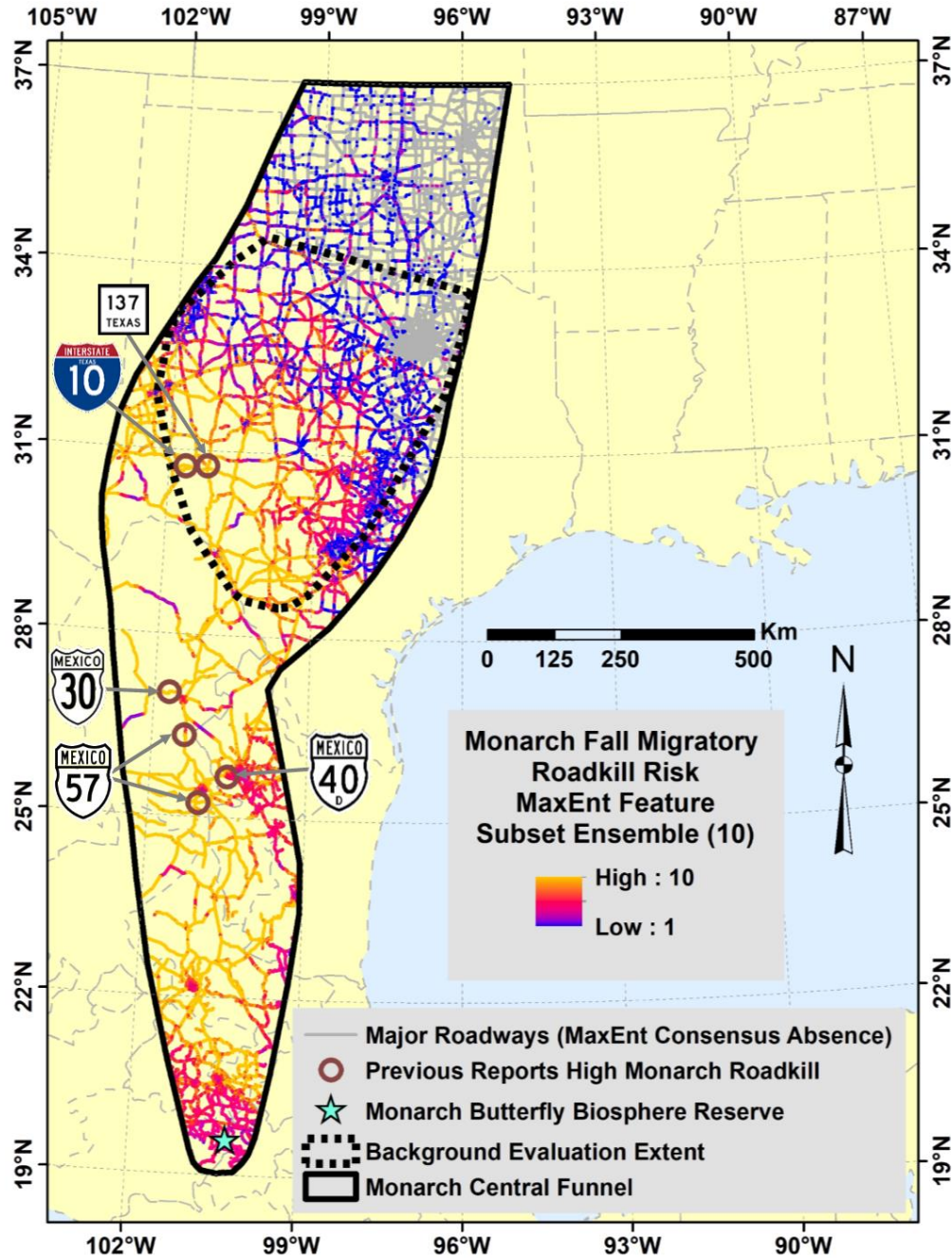


Figure 4. MaxEnt frequency consensus for feature subset ensemble of ten models developed from random subsets of ten of 20 variables correlated less than 0.7 (see Appendix Section 5 for embedded zipped shapefile of MaxEnt consensus model). [2 Columns; Color Figure Online]

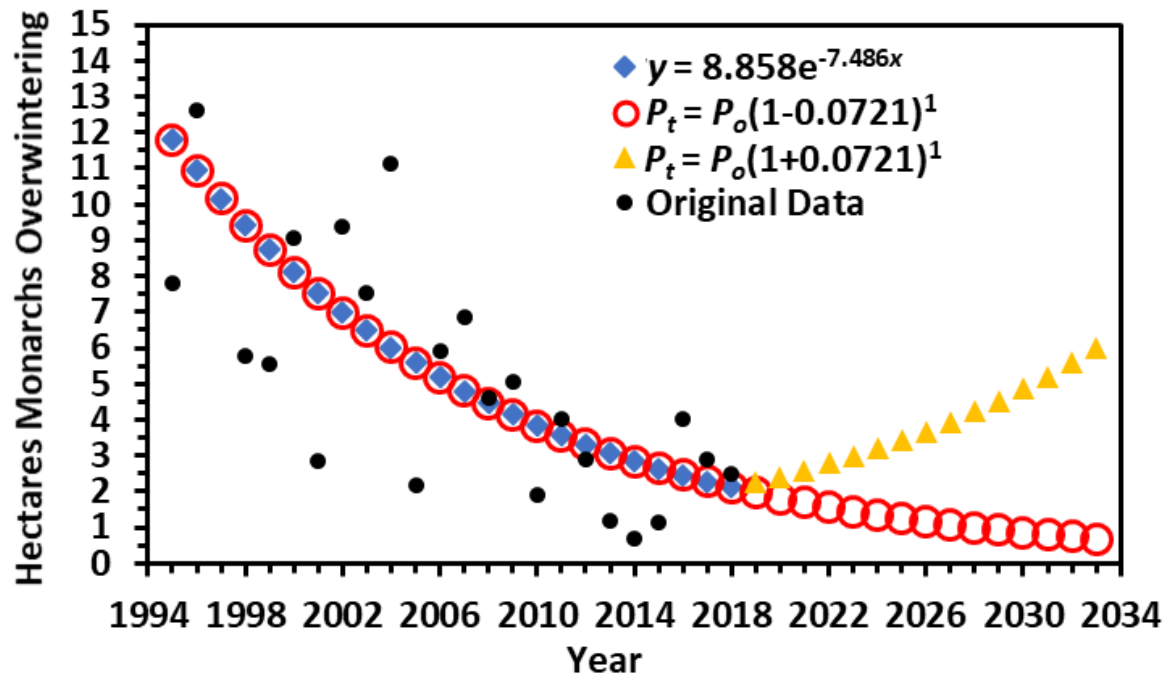


Figure 5. Annual monarch population in hectares in Mexican overwintering sites from 1995 to 2018 (original data, black circles; Vidal and Rendón-Salinas et al. 2014; Monarch Watch 2018b) with fitted exponential curve, $y = ae^{bx}$ (adjusted $R^2 = 0.49$; $P = 0.00009$; blue diamonds), and corresponding geometric population growth equation curve, $P_t = P_o(1 + r/n)^{nt}$, where P_t is the final hectares (2.11), P_o is the initial hectares (11.79), t is the number of years (23), n is the number of sub-periods (1), and r is the population growth (or declination) rate (derived population declination of 7.21% per year; open red circles). Fifteen years to restore 6 ha of overwintering monarchs based on totally reversing the current decline to 7.21% growth per year (gold triangles). [1 Column; Color Figure Online]

Table 1. Monarch roadkill estimates per year for 2016 to 2017 over the Sonora-Sheffield roadkill hotspot, Texas background evaluation extent (BE) and the Central Funnel (Figs. 1-2) from the field data by road type using simple extrapolation or MaxEnt model projections.

Year	Millions of Monarch Roadkill ^a						
	Simple Extrapolation					MaxEnt Model Extrapolations $\bar{X} \pm SD$ (n = 10) ^b	
	Hotspot Data Separated			Hotspot Data Included		Hotspot Data Included	
	Sonora-Sheffield Hotspot	Texas BEE	Central Funnel	Texas BEE	Central Funnel	Texas BEE	Central Funnel
2016							
Millions of Dead	0.074	0.768	1.570	1.663	3.554	2.050 \pm 0.760	3.041 \pm 0.743
% Overwintering Population ^c	0.09%	0.90%	1.82%	1.93%	4.03%	2.36 \pm 0.86%	3.46 \pm 0.82%
% Texas BEE Mortality	9.58%						
% Central Funnel Mortality	4.69%	48.95%		46.78%		66.48 \pm 14.79%	
2017							
Millions of Dead	0.005	0.492	1.042	0.533	1.131	0.738 \pm 0.272	1.084 \pm 0.258
% Overwintering Population ^c	0.01%	0.79%	1.67%	0.86%	1.81%	1.19 \pm 0.43%	1.73 \pm 0.41%
% Texas BEE Mortality	1.02%						
% Central Funnel Mortality	0.32%	31.34%		47.16%		68.86 \pm 14.18%	
2016-2017							
Millions of Dead	0.058	0.561	1.138	0.995	2.118	1.460 \pm 0.540	2.119 \pm 0.510
% Overwintering Population ^c	0.09%	0.91%	1.82%	1.59%	3.33%	1.96 \pm 0.71%	2.82 \pm 0.66%
% Texas BEE Mortality	10.25%						
% Central Funnel Mortality	3.66%	35.75%		27.99%		67.90 \pm 15.08%	

^aMean roadkill rates (roadkill/km/year) (Table A.2) by road type for extrapolations are calculated from transects in the Texas BEE and incorporate estimates for all road edges. Roadkill rates are multiplied by length of road in various areas to obtain extrapolations (see Table A.3 for road lengths used in simple road type extrapolation) (for all calculations, see Appendix A, section 5).

^bSee Table A.4 for individual MaxEnt model estimates.

^cBased on 84.61 (2016) and 61.4 (2017) million overwintering monarchs (Monarch Watch 2018b). Estimated from 21 million monarchs per hectares overwintering (Thogmartin et al. 2017).

Table 2. MaxEnt model variable permutation importance for 19 variables used in ten random sets of ten of the 20 variables in monarch roadkill models.

Variable ^a	Abbreviation	Permutation Importance ^b , Mean \pm SD
Human population density per km ² in 9 km radius (population/254.47 km ²)	<i>popden9kr</i>	44.2 \pm 3.8 (3)
Autumn quarterly mean monthly actual evapotranspiration/potential evapotranspiration \times 1000	<i>etrt_autq</i>	41.7 \pm 23.3 (2)
Annual mean monthly rainfall (mm)	<i>prec_ann</i>	36.4 \pm 15.6 (4)
Human population density per km ² in 3 km radius (population/28.27 km ²)	<i>popden3kr</i>	30.1 \pm 0.0 (1)
Elevation (m)	<i>elev</i>	27.9 \pm 5.5 (4)
Distance to urban areas (areas with \geq 300 humans per km ²) (km)	<i>urbdist</i>	27.1 \pm 10.8 (4)
Road density, km road in 3 km radius (km/28.27 km ²)	<i>roadden3kr</i>	19.6 \pm 2.4 (2)
Artificial surfaces % cover in 500 m radius	<i>artsur_500mr</i>	13.4 \pm 3.2 (3)
Autumn quarterly mean monthly maximum temperature ($^{\circ}$ Celsius)	<i>tmax_autq</i>	10.5 \pm 9.8 (3)
Distance to highways (m)	<i>hwydist</i>	10.4 \pm 2.5 (7)
Latitude (decimal degrees)	<i>latitude</i>	6.4 \pm 4.3 (3)
Grasslands % cover in 500 m radius	<i>grslnd_500mr</i>	5.6 \pm 3.4 (9)
Autumn mean quarterly wind speed (m/second)	<i>wndsp_autq</i>	5.6 \pm 5.4 (4)
Distance to secondary roads (m)	<i>secrddist</i>	5.1 \pm 2.9 (8)
Shrublands % cover in 500 m radius	<i>shrub_500mr</i>	4.5 \pm 3.4 (9)
Distance to primary roads (m)	<i>primrddist</i>	2.3 \pm 3.0 (4)
Road density, km road in 500 m radius (km/0.79 km ²)	<i>roadden500mr</i>	2.2 \pm 3.8 (5)
Distance to High Flow Accumulation Areas ($>60,000$ ~km ² upslope grid cells) (m)	<i>strmhiflodist</i>	1.2 \pm 1.0 (5)
Topographic Position Index (TPI), 3 km radius	<i>tpi3kr</i>	1.0 \pm 0.2 (5)
Cultivated land % cover in 500 m radius ^c	<i>cult_500mr</i>	0.0 \pm 0.0 (0)

^a See Table S1 for sources of variables. Variables ordered from highest to lowest mean permutation importance.

^b Permutation importance of the variable in the MaxEnt models, number of models in which the variable was used out of the 10 random models in parentheses.

^c Cultivated land layer was initially included in all 10 random models, but it was not included by MaxEnt in calculating any of the models.

APPENDIX D:
MANUSCRIPT SECTION II, SUPPLEMENT;
SPATIAL RISK ASSESSMENT OF EASTERN MONARCH BUTTERFLY
ROAD MORTALITY DURING AUTUMN MIGRATION WITHIN THE
SOUTHERN CORRIDOR

Biological Conservation – Supplement

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1. Spring 2017 Texas monarch roadkill survey

1.1 Methods

We conducted monarch roadkill surveys from 12 April to 21 May 2017 over much of the central-eastern portion of Texas approximately bounded by US highway 59 to the east, about 29°N to the south, about 101°W to the west and US highway 287 to the north (Fig. A.1). Surveys were conducted over four one-week periods progressing from south to north. Monarch roadkill counts were collected from one by 100 m transects along the edge of one side of the road following the described procedures for the fall 2016–2017 roadkill surveys.

1.2 Results

For the spring 2017 Texas roadkill survey, monarch road mortality was detected within only two of the 54 transects, and both detections were single males (Fig. A.1). One monarch roadkill was along Texas highway 7 east of Marlin on 28 April. The second monarch roadkill was along US highway 380 west of Runaway Bay on 10 May.

2. Monarch roadkill surveys and simple roadkill extrapolation

2.1 Methods

Two methods were used for extrapolating survey transect roadkill rates per km over larger areas: (1) “hotspot separated” extrapolation, for which roadkill rates from hotspot and non-hotspot regions were used to make separate extrapolations for each region; and (2) “hotspot included” extrapolation, where hotspot data was included with non-hotspot data for calculating roadkill rates and extrapolating over the entire region. The hotspot separated extrapolation allowed comparisons between roadkill in hotspot and non-hotspot areas, but this method assumed hotspots were absent when extrapolating beyond the study area, such as to Mexico, since non-hotspot roadkill rates are assigned to all new areas. The hotspot included extrapolation could not compare hotspot and non-hotspot areas, but assumed the potential for hotspots when extrapolating beyond the study area to Mexico, since hotspot data is included for the extrapolation.

For hotspot separated extrapolations, all of the hotspot transect data was utilized, but for hotspot included extrapolation, the hotspot data was thinned (or down-weighted) when the sampling effort in the hotspot area was higher than in non-hotspot area. For example, when calculating hotspot separated extrapolation for 2016, roadkill per km rates were derived from 15 highway hotspot transects for comparison with 22 highway non-hotspot transects. For hotspot included extrapolations for 2016, the 15 highway hotspot transects had to be randomly thinned to only two highway transects before inclusion with the 22 non-hotspot transects. Thinning to two 2016 highway hotspot transects was done in order to maintain the same sampling effort of about one transect every 30–40 km for highways in both the hotspot and non-hotspot regions (e.g., IH-20 non-hotspot highway). The roadkill per km values for each of the two random highway hotspot transects in 2016 represented an overall average of ten randomly selected highway hotspot transect pairs (red numbers in “2016” sheet of section 5 spreadsheet). The first member

of each of the ten hotspot transect pairs was randomly drawn without replacement and paired with the nearest northern edge transect that was at least 30 km distant. The three highway roadkill hotspot transects for 2017 were already proportional to the 10 non-hotspot highway transects and were directly averaged with non-hotspot highway transects for hotspot included extrapolation (see spreadsheet in section 5 below for calculations). The same procedures were followed for hotspot included extrapolations for MaxEnt model projections (see red numbers in “2016 Data NoZeros” sheet in section 5 spreadsheet).

2.2. Results

When including 2016 highway roadkill hotspot data with non-hotspot data for extrapolations, the average highway roadkill rate for the ten random pairs of hotspot transects was 549.17 (\pm 330.26 SD; range 79.17 to 1,101.13), which is lower than the overall average of 645.38 (\pm 446.92 SD; range 0 to 1,088.91) for the 15 highway hotspot roadkill transects for 2016 (Table A.2).

3. Texas monarch roadkill survey data

3.1 Fall 2016–2017 monarch roadkill survey raw data – unthinned (geographic; WGS84) for use in calculating roadkill per km in extrapolations



Fall2016_2017Monarc
hRoadkillData.xlsx

3.2 Fall 2016–2017 monarch roadkill survey raw data – thinned to 2 km and shifted to roadway for use in developing MaxEnt models (North America Albers Equal Area Conic, NAD83)



Fall2016_2017Monarc
hRoadkillThinned.xlsx

3.3 Spring 2017 monarch roadkill survey raw data – unthinned (geographic; WGS84)



Sprng2017MonarchR
oadkillData.xlsx

4. Texas and Mexico monarch roadkill data from other sources



MonarchRoadkillOthe
rData.xlsx

5. Texas monarch roadkill survey data and MaxEnt roadkill extrapolations



Fall2016_2017Monarc
hRoadkillDataExtrapol

6. MaxEnt roadkill consensus model

Below is an embedded zipped shapefile of the MaxEnt frequency consensus of the feature subset ensemble of ten monarch roadkill models developed from random subsets of ten of 20 variables (Fig. 4).



MonarchCentralFunnelRoadkillMaxEnt10ModeFSEConsensusSumShapefile..zip

7. Environmental variables

All environmental rasters were converted to and processed within the North American Albers Equal Area Conic projection (North American 1983 datum) to best preserve spatial relationships. The void-filled SRTM DEM was hydrologically processed before identifying streams with different levels of flow accumulation and calculating various topographic moisture indices. The percent cover of various Globeland30 (30 m resolution) land cover types (Chen et al. 2015) within a 500 m circular radius window size was calculated in ArcGIS (ESRI, Redlands, CA, USA). Road layers of the three major road types from 2017 were downloaded from Geofabrik (<http://download.geofabrik.de/>) and converted to rasters aligned with the DEM. Euclidean distances to urban areas, various road types, and stream layer flow accumulation categories were calculated in ArcGIS.

8. Correo Real roadkill report

The Correo Real (2015) Fall bulletin No. 15 from 8 November, 2015 is not available at the Correo Real (Royal Mail) Mexican monarch citizen science website, which only has publications since 2016 (<http://correoreal.org.mx/noticias/>). The original Spanish translation and a version with October 2015 Mexico roadkill reports roughly translated into English (in red) are embedded below. (If having trouble opening below embedded pdf files, make sure Adobe Reader is your default PDF handler. This can be done inside the Adobe Reader program by selecting the Edit menu, Preferences, General, then press button “Select as Default PDF Handler”, press “Continue” button).



CorreoReal2015_Mex
icoRoadkillSpanish.pdf



CorreoReal2015_Mex
icoRoadkillEnglishPart

9. Monarch population decline curve

The initial and final modeled number of hectares in the modeled standard exponential curve (Fig. 5) was used in a standard geometric population growth equation $P_t = P_o(1 + r/n)^{nt}$, where P_t is the final hectares (2.11), P_o is the initial hectares (11.79), t is the number of years (23), n is the number of sub-periods (1), and r is the population growth (or declination) rate. Solving for r yields an average -7.21% population decline per year over the last 23 years (see spreadsheet below for calculations).



MonarchPopulatio
nDeclineCurve.xlsx

Tables

Table A.1. Thirty environmental predictor indices (30.8 m resolution) evaluated for developing monarch roadkill models.

Variable Index	Abbreviation ^a	Source
<i>Six Road Indices</i> (based on three major road types of highways, primary roads, and secondary roads)		
Road density, km road in 500 m circular radius (km/0.78 km ²) ^b	<i>roadden500mr*</i>	Derived from OpenStreetMap (Geofabrik, 2017)
Road density, km road in 3 km circular radius (km/28 km ²) ^b	<i>roadden3kr*</i>	“
Distance to highways (motorways and trunks) (m)	<i>hwydist*</i>	“
Distance to primary roads (m)	<i>primrddist*</i>	“
Distance to secondary roads (m)	<i>secrddist*</i>	“
Traffic volume for 2015 (volume of vehicle traffic per year per road segment; Average Annual Daily Traffic; AADT)	<i>traffic_vol</i>	OpenStreetMap (Geofabrik, 2017) and Federal Highway Administration (2017)
<i>Three Human Population Density Indices</i>		
Human population density per km ² in 3 km circular radius (population/28 km ²) ^b	<i>popden3kr*</i>	Derived from CIESIN (2016)
Human population density per km ² in 9 km circular radius (population/254.5 km ²) ^b	<i>popden9kr*</i>	“
Distance to urban areas ≥ 300 humans per km (km)	<i>urbdist*</i>	“
<i>Nine Topographic Indices</i>		
Elevation (m)	<i>elev*</i>	NASA JPL (2013)
Topographic Position Index (TPI), 500 m circular radius ^c	<i>tpi500mr</i>	Derived from 1 arc second resolution SRTM elevation (NASA JPL 2013)
TPI, 3 km circular radius ^c	<i>tpi3kr*</i>	“
Compound Topographic Index (CTI) ^c	<i>cti</i>	“
Distance to Streams (STRMDIST) (m)	<i>strmdist</i>	“
Distance to Low Flow Accumulation Areas (100–5,000 ~km ² upslope grid cells ^d ; STRMLOFLODIST) (m)	<i>strmloflodist</i>	“
Distance to Medium Flow Accumulation Areas (5,000–60,000 ~km ² upslope grid cells ^d ; STRMMDFLODIST) (m)	<i>strmmdflodist</i>	“

Table A.1. Thirty environmental predictor indices (30.8 m resolution) evaluated for developing monarch roadkill models.

Variable Index	Abbreviation ^a	Source
Distance to High Flow Accumulation Areas (>60,000 ~km ² upslope grid cells ^d ; STRMHIFLODIST) (m)	<i>strmhiflodist</i> *	“
Latitude (decimal degrees)	<i>latitude</i> *	Derived using ArcGIS
<i>Eight Land Cover Indices</i> (percent cover in 500 m radius window; area/0.78km ²)		
Artificial surfaces	<i>artsur_500mr</i> *	Globeland30 (Chen et al. 2015)
Barren lands	<i>bare_500mr</i>	“
Cultivated land	<i>cult_500mr</i> *	“
Forests	<i>forest_500mr</i>	“
Grasslands	<i>grslnd_500mr</i> *	“
Shrublands	<i>shrub_500mr</i> *	“
Water bodies	<i>water_500mr</i>	“
Wetland	<i>wetlnd_500mr</i>	“
<i>Four Climatic Indices</i> ^e		
Autumn quarterly mean monthly maximum temperature (°Celsius)	<i>tmax_autq</i> *	for 1960–1990 derived from WorldClim (2017) of Hijmans et al. (2005)
Annual mean monthly rainfall (mm)	<i>prec_ann</i> *	“
Autumn quarterly mean monthly actual evapotranspiration/potential evapotranspiration × 1000	<i>etrt_autq</i> *	“
Autumn mean quarterly wind speed (m/second)	<i>wndsp_autq</i> *	for 1970–2000 derived from WorldClim2 (2017) of Fick and Hijmans (2017)

^aAsterisks indicate 20 of 30 variables selected for developing final MaxEnt monarch roadkill niche models.

^bVariables of different scales (radii) can perform differently in niche models (e.g., Bellamy and Altringham 2013).

^cCalculated using Geomorphometry and Gradient Metrics Toolbox for ArcGIS (Evans et al. 2014).

^dFlow accumulation for a grid cell is defined by the number of upslope cells from which water can be accumulated, as calculated by ArcGIS software Flow accumulation tool.

^eAutumn quarter includes October, November, and December.

Table A.2. Monarch roadkill/km per year for 2016 to 2017 over the Sonora-Sheffield roadkill hotspot, Texas background evaluation extent (BEE) and the Central Funnel.

Road Type	Roadkill/km per Year in Texas BEE ^a			
	Presence/Absence Rate for Simple Extrapolation (Zero Data Included)			Presence Only Rate for MaxEnt Extrapolation (No Zero Data)
	Hotspot Data Separated Sonora-Sheffield Hotspot	Outside Hotspot	All Data (Hotspot Data Included)	All Data (Hotspot Data Included)
2016				
Highway	645.38	12.64	57.34	169.59
Primary	-	10.75	10.75	26.45
Secondary	442.56	25.79	57.85	150.40
2017				
Highway	52.78	31.67	36.54	58.25
Primary	-	8.32	8.32	36.06
Secondary	-	6.15	6.15	28.30
2016-2017				
Highway	584.96	18.58	49.79	121.33
Primary	-	9.42	9.41	30.38
Secondary	71.26	12.88	24.82	89.35

^aThe mean roadkill rates are calculated from data in the BEE and incorporate estimates for all road edges (for calculations, see Appendix A, section 5).

Table A.3. Road length by road type for the Sonora-Sheffield roadkill hotspot, Texas background evaluation extent (BEE) and the Central Funnel used for simple extrapolation by road type.

Road Type	Road Length (km)		
	Sonora-Sheffield Hotspot	Texas BEE	Central Funnel
Highway	94.94	8,907	18,617
Primary	0.00	11,878	25,918
Secondary	27.82	17,705	38,169

Table A.4. Monarch roadkill estimates per year for 2016 to 2017 data over the Texas background evaluation extent (BEE) and the Central Funnel for each MaxEnt roadkill model according to estimated roadkill/km for lengths of predicted roadkill presence of each road type.^a

MaxEnt Model Number	Millions of Monarch Roadkill (Hotspot Data Included) ^a														
	2016					2017					2016-2017				
	Millions of Dead		Mortality as % Overwint. Pop.		Texas BEE as %	Millions of Dead		Mortality as % Overwint. Pop.		Texas BEE as %	Millions of Dead		Mortality as % Overwint. Pop.		Texas BEE as % Funnel
	Texas BEE	Central Funnel	Texas BEE	Central Funnel		Texas BEE	Central Funnel	Texas BEE	Central Funnel		Texas BEE	Central Funnel	Texas BEE	Central Funnel	
1	1.315	3.639	1.53%	4.12%	36.15%	0.488	1.271	0.79%	2.03%	38.40%	0.935	2.525	1.26%	3.34%	37.04%
2	2.728	3.751	3.12%	4.25%	72.73%	0.974	1.321	1.56%	2.11%	73.77%	1.940	2.602	2.59%	3.44%	74.56%
3	2.843	3.712	3.25%	4.20%	76.59%	0.991	1.284	1.59%	2.05%	77.19%	2.004	2.549	2.67%	3.37%	78.60%
4	2.733	3.732	3.13%	4.22%	73.23%	0.986	1.324	1.58%	2.11%	74.47%	1.949	2.598	2.60%	3.44%	75.01%
5	1.005	1.846	1.17%	2.14%	54.42%	0.358	0.664	0.58%	1.07%	53.92%	0.716	1.294	0.97%	1.74%	55.33%
6	1.684	2.570	1.95%	2.95%	65.49%	0.599	0.911	0.97%	1.46%	65.77%	1.198	1.795	1.61%	2.40%	66.75%
7	2.973	3.580	3.39%	4.06%	83.04%	1.079	1.312	1.73%	2.09%	82.24%	2.123	2.513	2.83%	3.33%	84.49%
8	2.294	2.731	2.64%	3.13%	84.01%	0.860	1.041	1.38%	1.67%	82.60%	1.652	1.932	2.21%	2.58%	85.50%
9	1.835	2.865	2.12%	3.28%	64.05%	0.655	1.011	1.05%	1.62%	64.75%	1.307	1.996	1.76%	2.66%	65.47%
10	1.095	1.986	1.28%	2.29%	55.12%	0.388	0.700	0.63%	1.13%	55.45%	0.779	1.384	1.06%	1.86%	56.25%
Mean	2.050	3.041	2.36%	3.46%	66.48%	0.738	1.084	1.19%	1.73%	66.86%	1.460	2.119	1.96%	2.82%	67.90%
SD	0.760	0.743	0.86%	0.82%	14.79%	0.272	0.258	0.43%	0.41%	14.18%	0.540	0.510	0.71%	0.66%	15.08%

^aBased upon multiplying length of road type by roadkill density per km for road type (presence only; see Table A.2) for each MaxEnt model (for all calculations, see Appendix A, section 5).

^bBased on 84.61 and 61.4 million monarchs overwintering in 2016 and 2017, respectively (Monarch Watch 2018b). Overwintering estimates were averaged for the combined 2016-2017 data. The value of 21.1 million monarchs per hectare overwintering was used in estimations (Thogmartin et al. 2017).

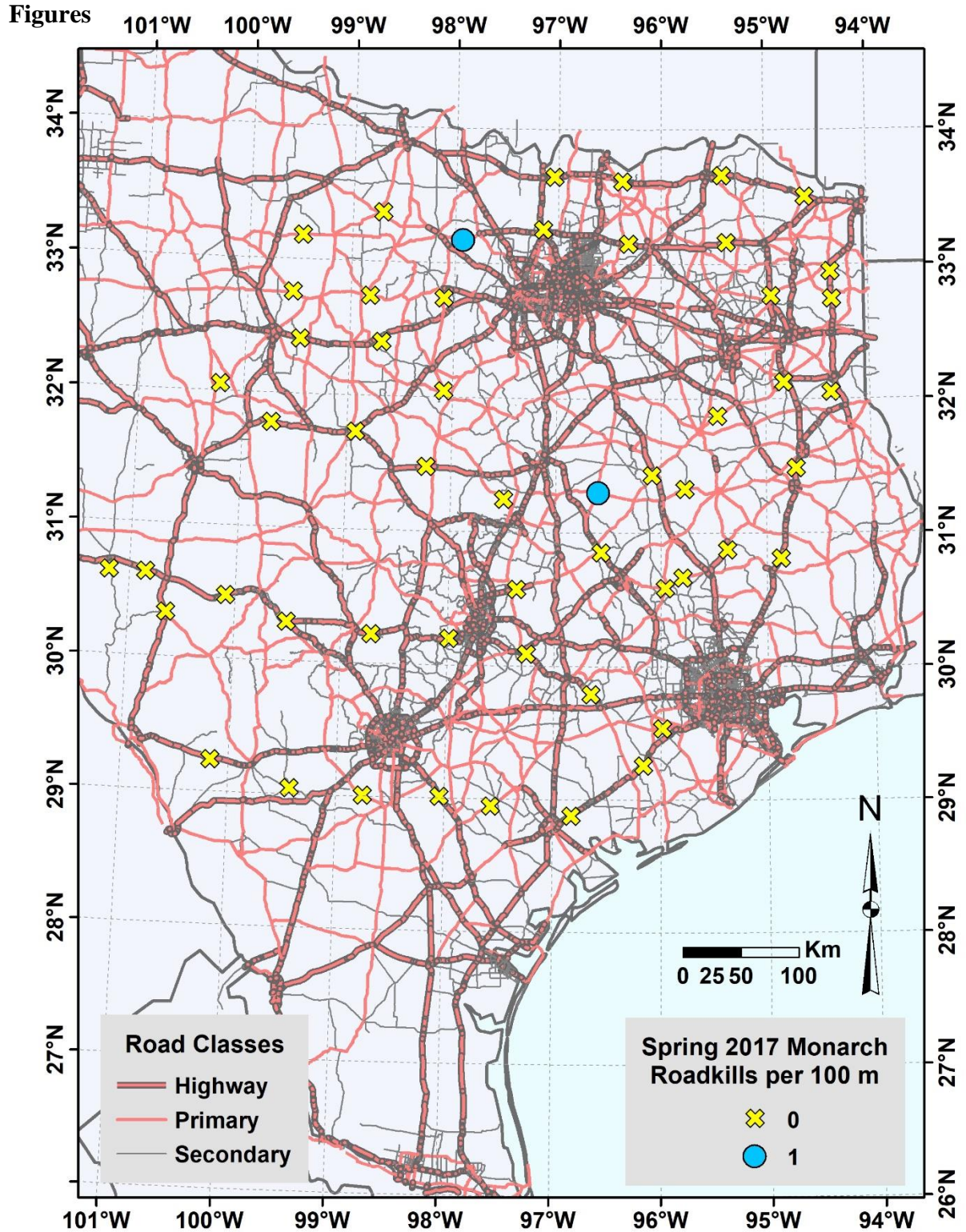


Figure A.1. Monarch roadkill spring 2017 survey results for 1 m by 100 m transects along major road classes within Texas.

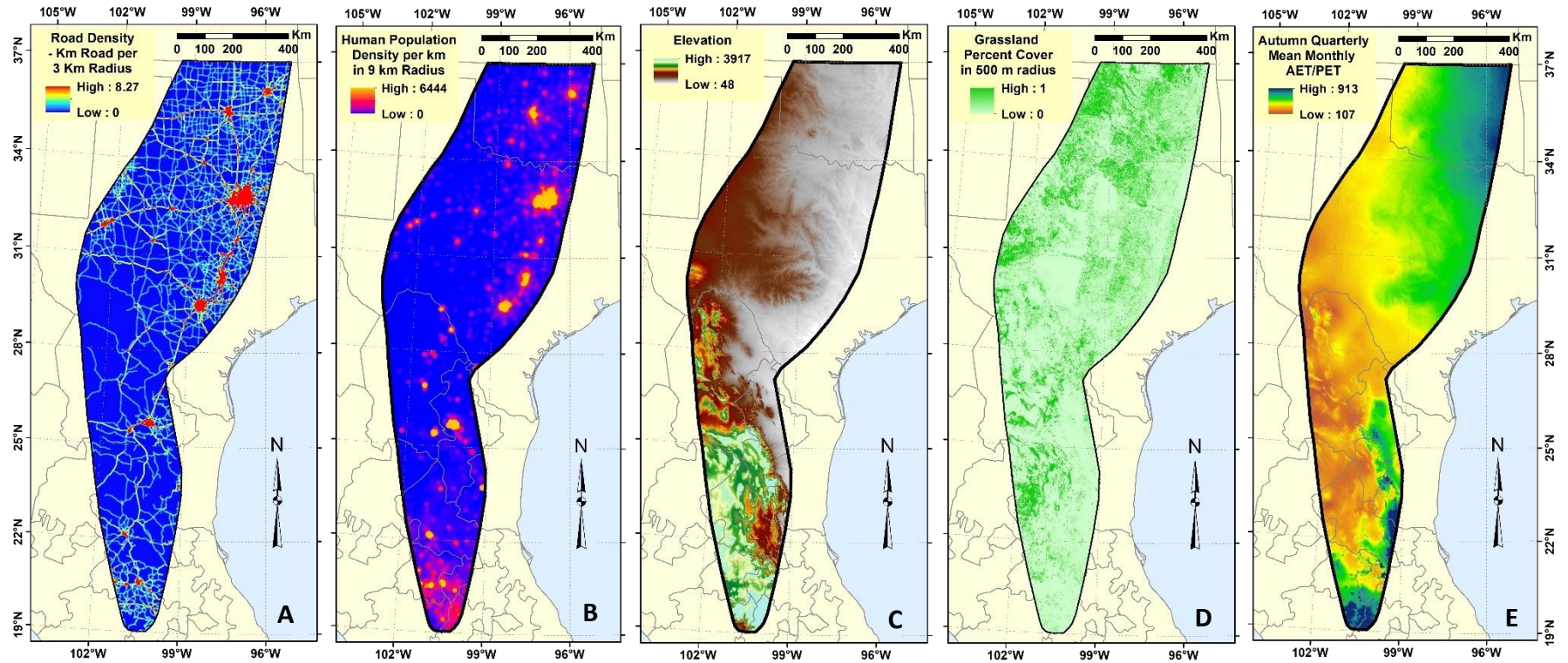


Figure A.2. Representative important environmental variables (30 m resolution) for five types of indices in MaxEnt niche models of monarch fall migratory roadkill within the Central Funnel: (A) road index of kilometers of road per three km radius (*roadden3kr*); (B) human population index of population density within a 9 km radius (*popden9kr*); (C) topographic index of elevation (*elev*); (D) land cover index of percent cover of grasslands in a 500 m radius (*grslnd_500mr*); and (E) climatic index of autumn quarterly mean monthly actual evapotranspiration (AET)/potential evapotranspiration (PET) \times 100 (see Table 2 for variable importance).

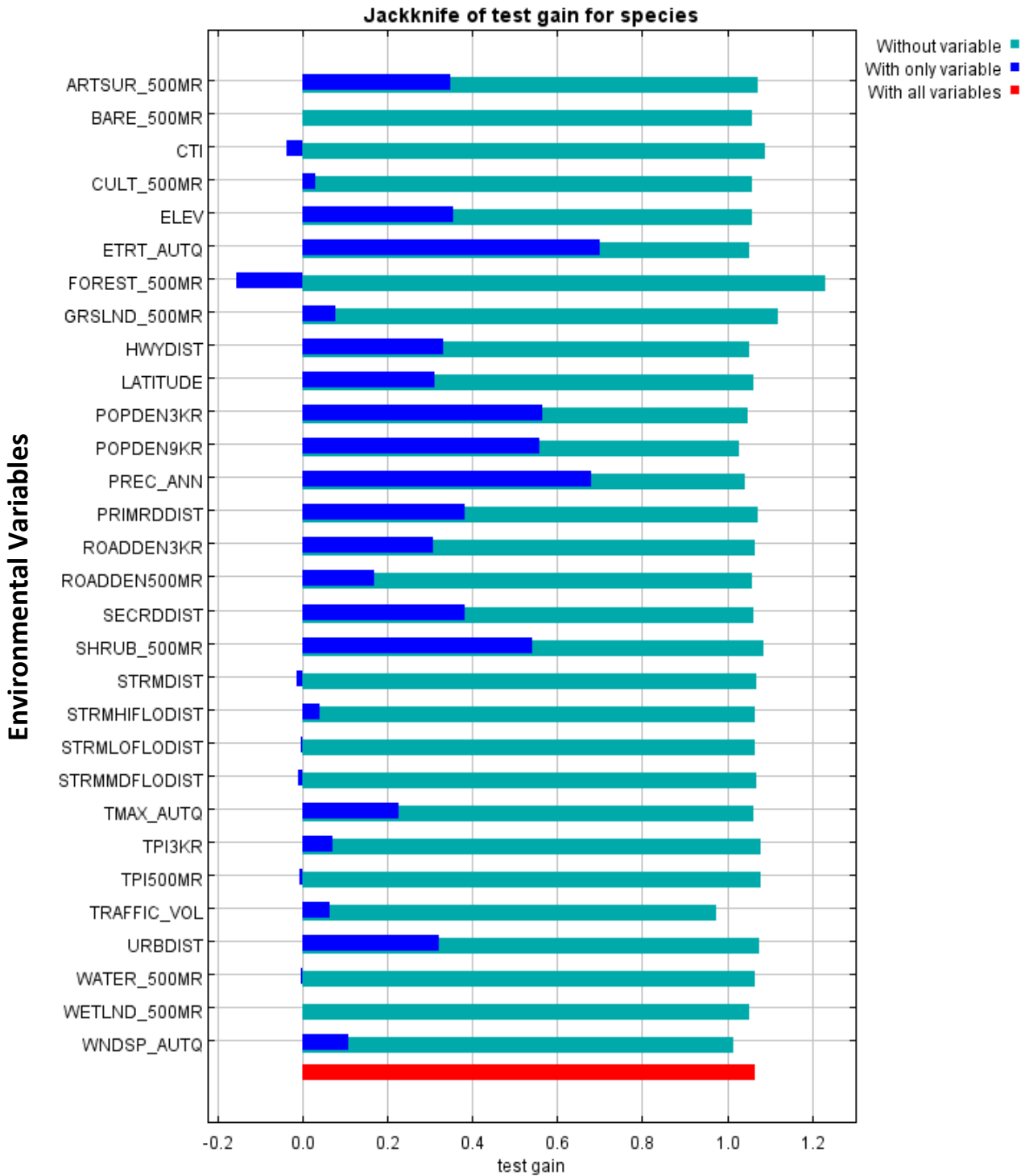


Figure A.3. MaxEnt variable importance in jackknife analysis of test gain for 30 total environmental variables in the monarch roadkill model (see Table A.1 for variable abbreviations).

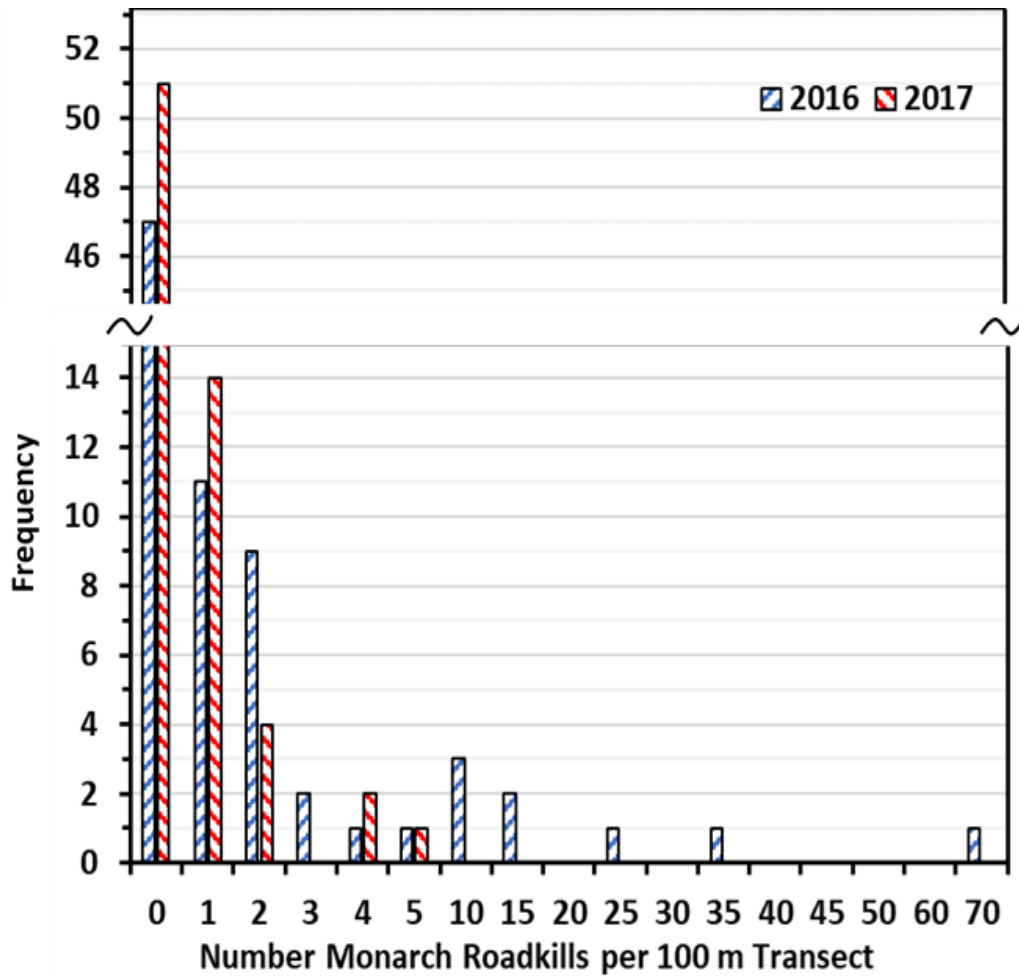


Figure A.4. Frequency distribution of monarch roadkill counts (spatially thinned to 2 km) for 1 m by 100 m transects for fall 2016 and 2017 along major road classes within the background evaluation extent of the monarch Central Funnel in Texas (Figs. 1-2).

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APPENDIX E:
SUPPLEMENT SECTION III;
MONARCH MILKWEED HABITAT MODELS AND LAND COVER RISK
ASSESSMENT

Methods

In calculating the milkweed species land cover utilization index (LCUI), milkweed locations were weighted to reduce bias from human population and nearness to roads according to land cover class. Each initial milkweed occurrence value of one was divided by the population density within a 20km radius for the 1 km resolution grid cell to yield *PopDenInd*. Maximum values of *PopDenInd* were limited to one, and minimum values could be near zero. We assumed that the percentage of milkweed locations reported within 20 meters of roads (near roads) was higher than for areas away from roads for all land cover classes. However, some land cover classes may have proportionally higher areas near roads, biasing the near road observations in favor of these land cover classes. In order to provide lower weight to milkweed observations near roads for land classes with more area near roads, we developed a road correction factor, *RoadCorrFactor*, to multiply with *PopDenInd* and down-weight the values where necessary. We first generated 10,000 random points within the milkweed core habitat area (MaxEnt minimum consensus model) and calculated the percentage of random points near the roads for each land cover class, *RandPercNearRoad*. For points near the road for each land class, we down-weighted the milkweed observations to the extent that the *RandPercNearRoad* value was higher for that land cover class compared to the minimum value of *RandPercNearRoad* among all land cover classes. Consequently, the value of *RoadCorrFactor* for a given land cover class was calculated as the minimum *RandomPercNearRoad* of all land cover classes divided by actual *RandomPercNearRoad* for the land cover class, $RoadCorrFactor = (\text{minimum } RandomPercNearRoad) / (\text{actual } RandomPercNearRoad)$. For instance, if the Developed, Low Intensity land cover class had the minimum *RandomPercNearRoad*, such as 0.027, and the *RandomPercNearRoad* for Developed, Open Space was 0.034, the *RoadCorrFactor* would be $0.027/0.034$, or 0.794. In contrast, the *RoadCorrFactor* for a land cover with a higher actual *RandomPercNearRoad*, such as for Evergreen Forest with 0.056, would be larger, equaling $0.027/0.056$, or 0.482, which provides a greater down-weighting correction. Since Evergreen Forest points near roads are potentially more common due to bias from the higher proportion of Evergreen Forest near roads, their locations near the road required more down-weighting than for other land cover classes which had lower distributions near the road, such as Developed Open Space. After multiplying the *PopDenInd* values near roads by the *RoadCorrFactor*, we obtained a road corrected population density index of *PopDenRoadInd*, representing the weighted value of the milkweed observation corrected by human population index and nearness to roads. These *PopDenRoadInd* values were summed for each land cover class for calculating the percentage of milkweed observations per land class (% land cover utilized). This value was then divided by the percent land cover available for a given class (% land cover available) to obtain the LCUI. The percent land cover available per class was obtained from zonal statistics of the 30 m resolution 2011 NLCD layer clipped to the 1 km resolution milkweed core habitat within the South-Central

US region. Embedded files of more detailed procedures and scripts in ArcPython (for ArcGIS software) and R for calculating LCUI for the land cover risk assessment are provided below (Table E.2).

Tables

Table E1. Ninety-five environmental predictor indices (1 km resolution) used in developing 12 selected MaxEnt niche models for four *Asclepias* species with six to eight of 95 variables.^a

Variable Index (Source)	Grid Name Abbreviation
57 Climatic Indices (for 1960–1990 derived from WorldClim [2014] of Hijmans et al. [2005])	
19 Bioclim Indices (WorldClim 2014)	
Annual mean temperature	<i>bio_1</i>
Mean diurnal range (mean of monthly TMAX – TMIN)	<i>bio_2</i>
Isothermality (<i>bio_2/bio_7</i>) ($\times 100$)	<i>bio_3</i>
Temperature seasonality (standard deviation $\times 100$)	<i>bio_4</i>
Maximum temperature of warmest month	<i>bio_5</i>
Minimum temperature of coldest month	<i>bio_6</i>
Temperature annual range (<i>bio_5 – bio_6</i>)	<i>bio_7</i>
Mean temperature of wettest quarter	<i>bio_8</i>
Mean temperature of driest quarter	<i>bio_9</i>
Mean temperature of warmest quarter	<i>bio_10</i>
Mean temperature of coldest quarter	<i>bio_11</i>
Annual precipitation	<i>bio_12</i>
Precipitation of wettest month	<i>bio_13</i>
Precipitation of driest month	<i>bio_14</i>
Precipitation seasonality (coefficient of variation)	<i>bio_15</i>
Precipitation of wettest quarter	<i>bio_16</i>
Precipitation of driest quarter	<i>bio_17</i>
Precipitation of warmest quarter	<i>bio_18</i>
Precipitation of coldest quarter	<i>bio_19</i>
Subtotal	
19 Supplementary Climatic (SuppClim) Indices^{a,b}	
Annual mean minimum temp. of coldest month (TMIN)	<i>tmin_ann</i> <i>tmin_winq</i> , <i>tmin_sprq</i> , <i>tmin_sumq</i> ,
Quarterly mean monthly minimum temperature (4)	<i>tmin_autq</i>
Annual mean maximum temp. of warmest month (TMAX)	<i>tmax_ann</i>

Table E1 (cont.). Ninety-five environmental predictor indices (1 km resolution) used in developing 12 selected MaxEnt niche models for four *Asclepias* species with six to eight of 95 variables.^a

Quarterly mean monthly maximum temperature (4)	<i>tmax_winq,</i> <i>tmax_sprq,</i> <i>tmax_sumq,</i> <i>tmax_autq</i>
Annual mean monthly rainfall (P) (mm)	<i>prec_ann</i> <i>prec_winq,</i> <i>prec_sprq,</i> <i>prec_sumq,</i> <i>prec_autq</i>
Quarterly mean monthly rainfall (50%) (4)	
Effective Warmth Index (from mean monthly temperatures \times 10 above 5°C)	<i>ew_indx</i>
Rivas-Martinez (RM) ombrothermic index (from monthly MTMP and P)	<i>ombro_index</i>
RM continentality index (TMAX – TMIN) \times 10 (CONT)	<i>cont_index</i>
RM thermicity index (MTMP + TMX_COLD + TMN_COLD) \times 10	<i>therm_index</i>
<i>19 Actual and Potential Evapotranspiration (AET-PET) Indices</i> (PET; Zomer et al., 2007; 2008; AET; Trabucco and Zomer, 2010) ^b	
Total annual reference evapotranspiration from Hargreaves model (PETH) (mm)	<i>tpeth_ann</i> <i>peth_winq,</i> <i>peth_sprq,</i> <i>peth_sumq,</i> <i>peth_autq</i>
Quarterly mean monthly PETH (4)	
Thornwaite summer concentration thermal efficiency (summer PETH/annual PETH) \times 1000	<i>tpeths_tpetha</i>
Willmott and Feddema climate moisture index (from total annual PETH and PREC) \times 1000	<i>im_index</i>
Total annual actual evapotranspiration from Thornwaite-Mather water balance model (TMWBM) (AETT) (mm)	<i>taett_tann</i> <i>aett_winq, aett_sprq,</i> <i>aett_sumq, aett_autq</i>
Quarterly mean monthly AETT (4)	
Total annual evapotranspiration ratio (AETT/PETH) \times 10	<i>etrt_ann</i> <i>etrt_winq, etrt_sprq,</i> <i>etrt_sumq, etrt_autq</i>
Quarterly mean monthly AETT/PETH (4) \times 1000	
Modified Driscoll-Yee Fong Continentality index ^c	<i>cont_dfmo</i>
Climate water deficit (<i>tpeth_ann</i> – <i>taett_tann</i>)	<i>cwd_ann</i>
<i>14 Topographic Indices</i>	
<i>Ten Geomorphologic Indices</i> (derived from 15 arc second resolution HydroSHEDs grids of Lehner et al. 2008; last eight indices calculated using Geomorphometry and Gradient Metrics Toolbox for ArcGIS [Evans et al., 2014]) ^b	
Elevation	<i>elev</i>

Table E1 (cont.). Ninety-five environmental predictor indices (1 km resolution) used in developing 12 selected MaxEnt niche models for four *Asclepias* species with six to eight of 95 variables.^a

Slope	<i>slope</i>
Topographic Position Index (TPI), 3 km circular radius	<i>tpi3kr</i>
TPI, 19 km circular radius	<i>tpi19kr</i>
Elevation Relief Ratio (ERR, = Surface Relief Ratio, SRR), 3 km circular radius	<i>err3kr</i>
Compound Topographic Index (CTI)	<i>cti</i>
Heat Load Index (HLI)	<i>hli</i>
Integrated Moisture Index (IMI)	<i>imi</i>
Site Exposure Index (SEI)	<i>sei</i>
Slope Cosine Aspect Index (SCAI)	<i>scai</i>
<i>Four Hydrogeomorphologic Indices</i> (derived from 15 arc second resolution HydroSHEDs polyline river network shapefile of Lehner et al. 2008)	
Distance to Streams (STRMDIST)	<i>strmdist</i>
Distance to Low Flow Accumulation Areas (< 5,000 cells; STRMLOFLODIST)	<i>strmloflodist</i>
Distance to Medium Flow Accumulation Areas (5,000–60,000 cells; STRMMDFLODIST)	<i>strmmdflodist</i>
Distance to High Flow Accumulation Areas (>60,000 cells; STRMHIFLODIST)	<i>strmhiflodist</i>
24 Edaphic Indices (derived from 250 m resolution global SoilGrids250m of Hengl et al. [2017])	
12 Soil Property Indices	
<i>Top Soil: 0–5 cm</i>	
% Sand	<i>sand5cm</i>
% Clay	<i>clay5cm</i>
% Silt	<i>silt5cm</i>
Bulk Density	<i>blkden5cm</i>
Coarse Fragments	<i>frags5cm</i>
Soil pH in H ₂ O	<i>pH5cm</i>
<i>Deep Soil: 0–30 cm</i>	
% Sand	<i>sand30cm</i>
% Clay	<i>clay30cm</i>
% Silt	<i>silt30cm</i>
Bulk Density	<i>blkden30cm</i>
Coarse Fragments	<i>frags30cm</i>
Soil pH in H ₂ O	<i>silt30cm</i>
12 USDA Soil Classification Suborder Indices (six orders; percent probability per grid cell) ^d	
<i>Alfisols Order</i>	
Aqualfs	<i>aqualfs</i>
Udalfs	<i>udalfs</i>
Ustalfs	<i>ustalfs</i>

Table E1 (cont.). Ninety-five environmental predictor indices (1 km resolution) used in developing 12 selected MaxEnt niche models for four *Asclepias* species with six to eight of 95 variables.^a

<i>Aridisols Order</i>	
Argids	<i>argids</i>
Calcids	<i>calcids</i>
<i>Entisols Order</i>	
Orthents	<i>orthents</i>
Psamments	<i>psamments</i>
<i>Inceptisols Order</i>	
Ustepts	<i>ustepts</i>
<i>Mollisols Order</i>	
Udolls	<i>udolls</i>
Ustolls	<i>ustolls</i>
<i>Ultisols Order</i>	
Udults	<i>udults</i>
<i>Vertisols Order</i>	
Usterts	<i>usterts</i>

^aQuarters: winter- Jan, Feb, Mar; spring – Apr, May, Jun; summer – Jul, Aug, Sep; autumn – Oct, Nov, Dec. PREC = precipitation; TMAX = mean temperature of warmest month; TMIN = mean temperature of coldest month; TMAX_COLD = mean maximum temperature of coldest month; TMN_COLD = mean minimum temperature of coldest month.

^bFor additional sources of indices and details see Tracy et al. (2018).

^cIncorporates moisture correction factor of AET/PET × 10.

^dCommon soil suborders in south-central USA and northern Mexico.

Table E2. Procedures with ArcPython and R scripts, Excel charts, and ArcMap projects for developing species land cover risk assessment with the land cover utilization index (LCUI).











<i>Detailed Procedures (Word Document)</i>	
LandCoverRiskAssessmentProtocol_2Jan2019.docx	 LandCoverRiskAssessmentProtocol_2Jan20
<i>R Scripts</i>	
LandCoverRiskAssessment_NLCDPerceCoveriNatHiResMaxEntFSELoo p.R	 LandCoverRiskAssessment_NLCDPercentCo
SpatialFilter_Function.R	 SpatialFilter_Function. R
EvalStatVars_Summary_Function.R	 EvalStat_Summary_Fu nction.R
Round2_Function.R	 Round2_Function.R
GamesHowell.Test.Padj_Function.R	 GamesHowell.Test.Pa dj_Function.R
<i>ArcPython Scripts</i>	
LandCoverRiskAssessment_PointandMaxEntModelNLCDProcessing.py	 LandCoverRiskAssessment_PointandMaxEnt
LandCoverRiskAssessment_ReclassifyLandCoverUtilizationIndex.py	 LandCoverRiskAssessment_PointandMaxEnt
<i>Excel Spreadsheet Data and Charts</i>	
A_oeno_20kPopDenRoadIndex_MaxEntModel_NLCDHazardSummarySta ts_15m.xlsx	 A_oeno_20kPopDenR oadIndex_MaxEntMoc
<i>ArcMap Project Map Packages (ArcGIS 10.3)</i>	
LandCoverRiskAssessmentMap_AoenNoLegends.mpk	 LandCoverRiskAssessmentMap_AoenNoLeg

Table E2 (cont.). Procedures with ArcPython and R scripts, Excel charts, and ArcMap projects for developing species land cover risk assessment with the land cover utilization index (LCUI).



LandCoverRiskAssess
mentMap_AoenLegen

LandCoverRiskAssessmentMap_AoenLegends.mpk

Figures

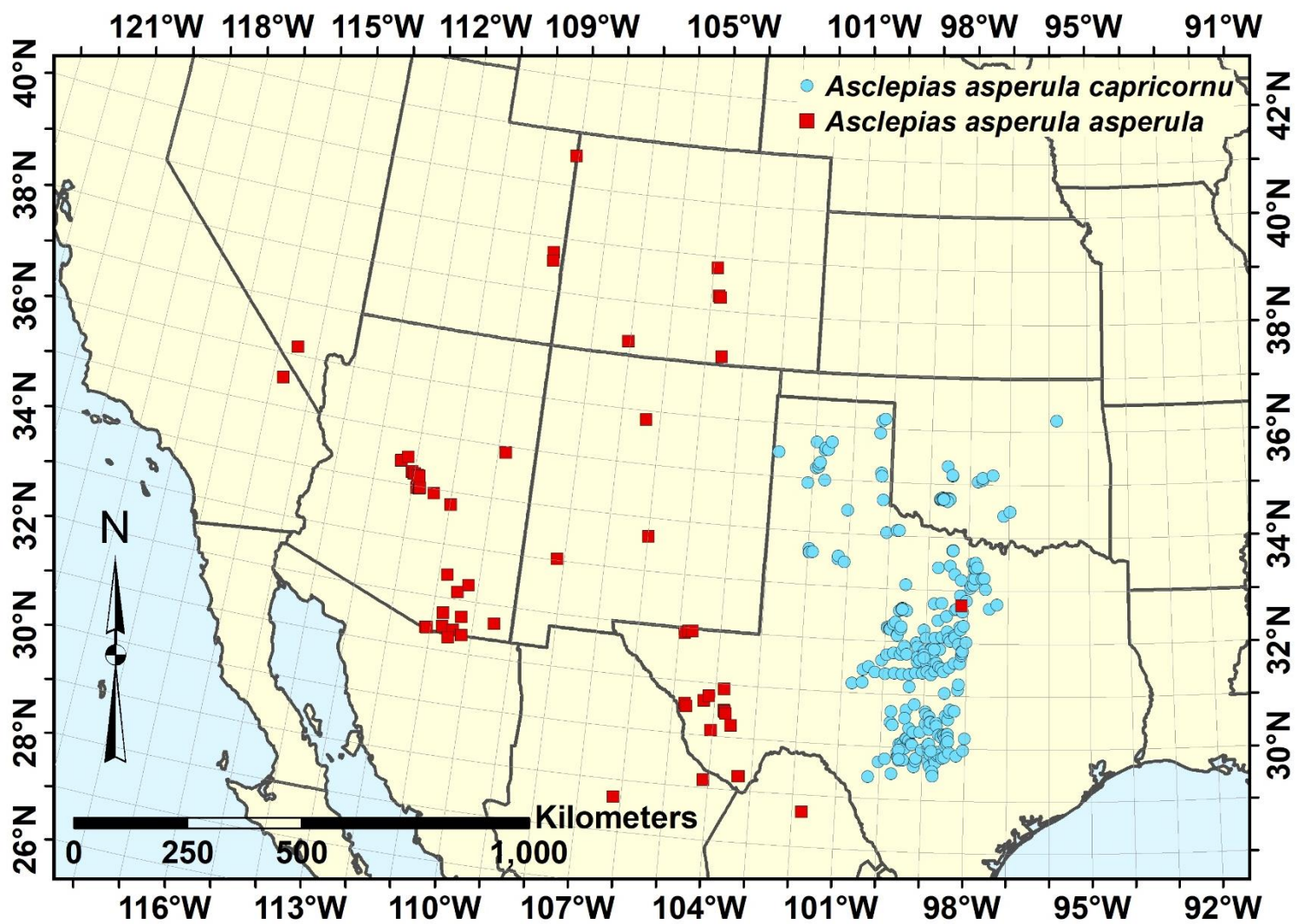


Fig. E.1. Distribution of subspecies *Asperula asperula capricornu* and *A. a. asperula* from identification using images from iNaturalist (2018).

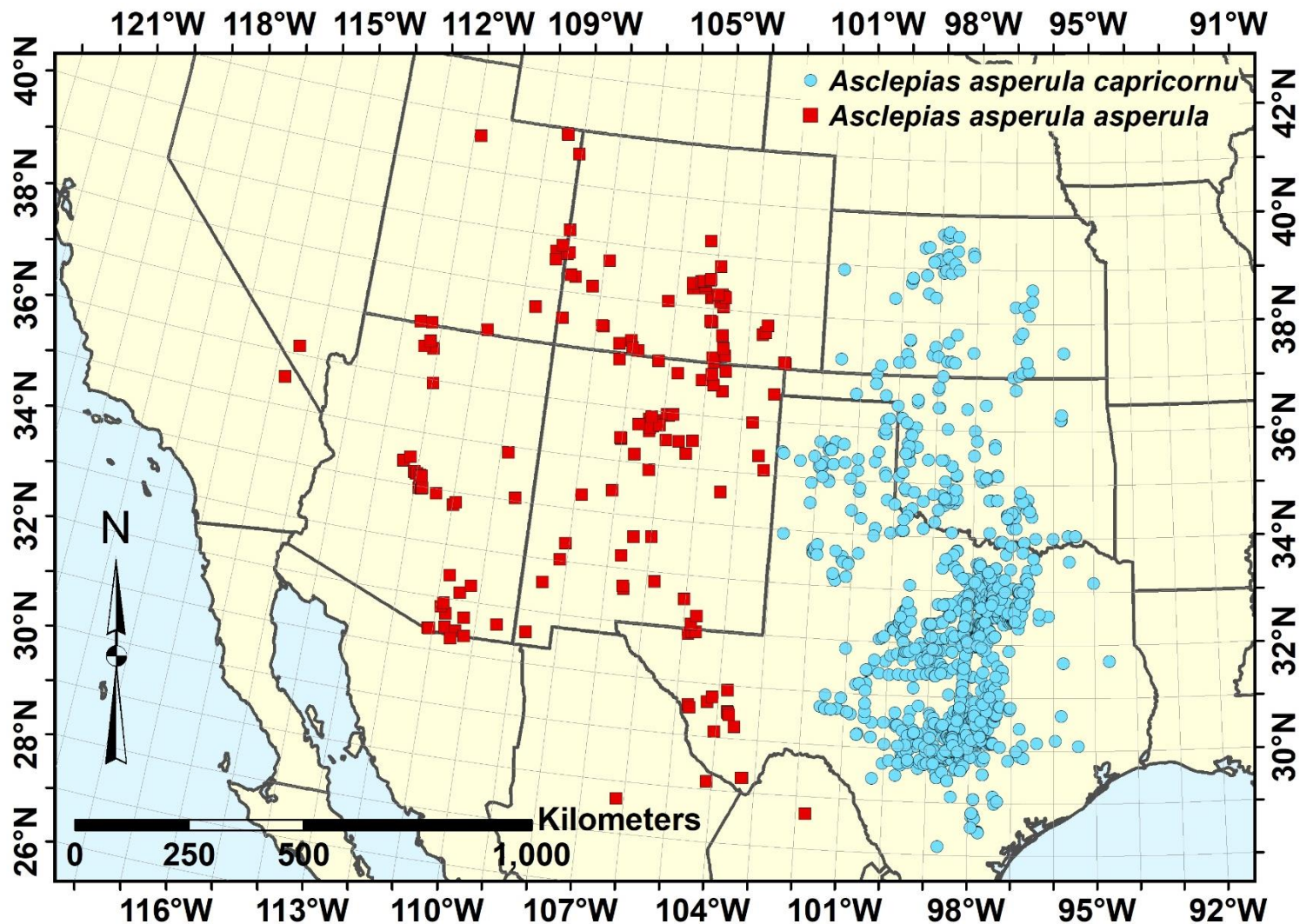


Fig. E.2. Distribution of subspecies *Asperula asperula capricornu* and *A. a. asperula* from identification using images from iNaturalist (2018) and subspecies identifications from GBIF (2018) and SeiNET (2018) that fall within subspecies boundaries established from Fig. E.1 consisting of Pecos River in Texas and 103°N along Texas and Oklahoma panhandles.